CXXIV. THE PRODUCTION OF ORGANIC COMPOUNDS OF SULPHUR IN BACTERIAL CULTURES WITH SPECIAL REFERENCE TO GLUTATHIONE.

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THERE is an extensive literature which deals with the production of sulphides by bacteria. Without attempting a detailed review of this literature it will be sufficient to draw attention to some of the more important facts which have been established.

It seems to be clear that a number of bacteria are able to produce H_2S from media containing sulphur, sulphite or cystine; but that H_2S is rarely produced from taurine or sulphates. Further, the importance of cystine as a source of sulphide has been demonstrated. Sulphide is produced when bacteria are cultivated in synthetic media containing cystine but no other source of sulphur [Sasaki and Otsuka, 1912; Myers, 1920]. As sources of sulphide the different peptones appear to vary considerably and even from sample to sample, when used in culture media for the demonstration of sulphide production by bacteria. Certain results, however, have been constantly obtained with different media and by different observers; such are active sulphide production by *B. paratyphosus* B., *B. enteritidis*, *B. proteus*, *B. cloacae*, *B. typhosus* and absence of sulphide production by *B. paratyphosus* A and the dysentery bacilli [Myers, 1920; Thompson, 1921; Tilley, 1923]. The formation of volatile sulphides by anaerobes has been frequently noted [Med. Res. Council, 1919].

It is interesting that Thompson observes a general although not invariable rule that bacteria in the coliform group which ferment lactose do not form sulphide. Tanner notes that yeasts possess much greater power than bacteria for reducing both organic and inorganic compounds of sulphur.

The observations with regard to the formation of organic compounds of sulphur by bacteria are not numerous. Sasaki and Otsuka [1912], working with pure cultures and synthetic media, were unable to demonstrate mercaptan formation. However, Kondo [1923], working with synthetic media containing *l*-cystine, showed that *B. proteus* constantly and *B. coli* occasionally produced mercaptan provided one of the following were present in the medium glucose, lactose, saccharose, glycerol or histidine. No other amino acid tested could take the place of histidine. He also obtained presumptive evidence of the production of ethyl sulphide or some other alkyl sulphide. H_2S was invariably produced under these conditions.

In view of the rather scanty existing evidence for the production of organic compounds of sulphur by bacteria it was of particular interest that Hopkins in the account published in 1921 of his important discovery of the compound glutathione referred to its probable occurrence in bacterial cultures. These observations were limited to the occurrence of the nitroprusside reaction in cultures of such bacteria as he had tested. At all events no mention is made of the isolation of the compound from bacterial cultures.

It was considered probable by Hopkins [1921; Hopkins and Dixon, 1922] that traces of H_2O_2 were formed in the course of oxidations in which glutathione acted as an accelerator of respiration in the presence of oxygen.

As we were engaged about that time in an investigation of the production of H_2O_2 by bacteria [McLeod and Gordon, 1922], it seemed to us to be desirable to get more extended information with regard to the occurrence of substances reacting with nitroprusside in bacterial cultures. We hoped to establish some correlation between production by bacteria of H_2O_2 on the one hand and of substances reacting with nitroprusside on the other.

The method of carrying out the reaction was as follows: a test-tube was filled to the depth of half an inch with $(\text{NH}_4)_2\text{SO}_4$ crystals; then 10 cc. of broth culture, 0.5 cc. of a 5 % solution of nitroprusside and lastly 5-7 cc. of strong NH_4OH were added.

All the cultures of anaerobes tested gave strong reactions and in so far as could be judged with a method, which is not particularly well adapted to quantitative determinations, the reactions with the more strict anaerobes appeared to be the stronger.

In observations with cultures of bacteria which could grow aerobically tests were made both with and without previous reduction. Reduction was effected by an aluminium mercury couple.

The strongest positive reactions were obtained with cultures of *B. paratyphosus* B. and of the cholera vibrio, weaker positives were obtained with *B. gaertner* and other strains allied to *B. paratyphosus* B. and with some strains of *B. coli*, with *B. typhosus* and with *B. proteus*. Doubtful results were got with some strains of *Staphylococcus aureus*, negative with others. Reactions were invariably negative with *Gonococcus*, *Meningococcus*, *Pneumococcus* and strains of Influenza bacilli, *Streptococci* (haemolytic and non-haemolytic), *Staphylococcus albus*, Flexner, Y, and Shiga strains of dysentery bacilli and with *B. paratyphosus* A. The nitroprusside reactions obtained with cultures of anaerobes or with cultures of *B. paratyphosus* B. grown under anaerobic conditions were apparently a good deal stronger than those got on reducing the original broth for 5-10 minutes with an active HgAl couple with a view to reducing the oxidised glutathione from the meat extract. Some part of this reaction may be due to inorganic sulphides, mercaptans or acetone but inasmuch as the reaction is little or not at all decreased by prolonged boiling, such compounds can only account for a small fraction of the reacting substances.

It was found, however, that a heavy suspension of a surface growth of *B. welchii* on a solid medium, whether boiled or unboiled, fresh or reduced, never gave a definite nitroprusside reaction.

This and some experimental work in which old and recent yeast extracts were compared with bacterial cultures in regard to rates of disappearance of the nitroprusside reaction led us to wonder whether the nitroprusside reaction occurring in bacterial cultures was not entirely due to a very effective reduction of the oxidised glutathione originally present in the medium.

The accuracy of this supposition was tested in two ways; the first was an investigation of the power of bacteria to produce a nitroprusside reaction in broth freed from substances reacting with nitroprusside. This substance, presumably glutathione, can be destroyed by heating with 1-2 parts per 1000 of H_2O_2 . It was not often possible however to destroy the residual H_2O_2 completely by subsequent boiling of the broth. R. Wolfenstein [1894] draws attention to the fact that the liability of H_2O_2 to destruction by heating has been exaggerated and shows that in the absence of (a) traces of heavy metals, (b) substances of alkaline reaction, and (c) solid particles of any kind, it is relatively heat stable. We therefore had recourse to three different methods of getting rid of the residual H_2O_2 . These were:

(1) Shaking with MnO_2 powder, the MnO_2 being subsequently removed by filtration.

(2) Warming with 0.5-1 % of glucose in the presence of a trace of FeSO_4 .

(3) Shaking with a heavy emulsion of staphylococci rich in catalase [McLeod and Gordon, 1923] and filtering off the staphylococci by means of a porcelain candle (Maassen type).

The broth resulting from each of those three forms of treatment was proved to be free from glutathione as it gave no nitroprusside reaction after prolonged reduction, $\frac{3}{4}$ -1 hour, with an active couple.

Both *B. welchii* and *B. paratyphosus* B. were successfully cultivated in all three media although the growths were distinctly less copious than in the original broths. Such cultures always failed to give any trace of nitroprusside reaction. Cultures of these bacteria in the same broth subjected to the same treatment with staphylococci but not oxidised by H_2O_2 gave frank nitroprusside reactions.

The second way of testing our theory was to observe whether by means of prolonged reductions by aluminium mercury couples it was possible to produce reactions in ordinary meat extract broth similar in intensity to those observed in bacterial cultures. The reductions to which reference is made in the earlier part of the paper were not carried out for more than 10-15 minutes. It was found that broth reduced for 1 hour by an active couple gave a reaction little if at all inferior to that obtained in the same broth after the growth of an anaerobe. We have therefore been led to conclude that bacteria do not produce glutathione when cultivated in nutrient broth and that the nitroprusside reactions observed in bacterial cultures are mainly or entirely due to the

special aptitude of the bacteria concerned for reducing the residual oxidised glutathione present in the medium. The nitroprusside reaction in a bacterial culture in meat extract broth is therefore an indication of the reducing powers of that bacterium and very possibly an indication of its capacity to utilise glutathione in carrying out some of the oxidations required for its metabolism.

Glutathione would not appear to play any part in the production of H_2O_2 by bacteria of the streptococcal group although it may in the case of the anaerobes. The last subject we hope to deal with in a subsequent communication. A point of some importance arising from these observations is the marked permanence of glutathione in the oxidised condition in media prepared from meat extract and the possibility that it is one of the important constituents of meat extract for promoting the growth of certain bacteria.

SUMMARY.

(1) A distinct nitroprusside reaction has been observed in 24–48 hour cultures of the following bacteria—all anaerobes tested (six, including *B. welchii*, *B. tetani* and *Vibrion septique*), *B. paratyphosus* B. and most allied species, *Cholera vibrio* (one strain), *B. typhosus*, *B. pyocyaneus*, some strains of *B. coli* and *B. proteus*.

(2) No distinct nitroprusside reactions have been observed in cultures of *Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Gonococcus*, *Meningococcus*, *B. influenzae*, *B. Morax-Axenfeld*, *B. paratyphosus* A. or of the dysentery bacilli (Flexner, Y, and Shiga types).

(3) Where a facultative anaerobe gives a nitroprusside reaction it is much stronger if the bacterium is grown in anaerobic or nearly anaerobic conditions.

(4) The thermo-stable substance reacting with nitroprusside detected in bacterial cultures is not produced by the bacteria but is the oxidised glutathione or some related compound originally present in the medium and reduced by the bacteria in the course of their growth.

(5) Bacteria therefore do not produce glutathione or substances allied to it reacting with nitroprusside.

(6) Bacteria capable of reducing glutathione probably utilise it when growing under anaerobic conditions to accelerate the oxidations necessary to their metabolism.

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