THE PRODUCTION OF METHYL MERCAPTAN BY FECAL BACTERIA GROWN ON A PEPTONE MEDIUM.

By C. A. HERTER.

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It is surprising that almost no systematic study has been devoted to methyl mercaptan, a common product of the putrefactive cleavage of proteid, in its relation to intestinal putrefaction. Nencki, who discovered this substance among the products of putrefaction, obtained a small amount of it from a large quantity of human feces, and reached the conclusion that it is a usual product of intestinal putrefaction. This conclusion is probably correct, although, strictly speaking, it does not appear fully justified by Nencki's observations, for the reason that the material used by him could not have been sufficiently fresh to exclude mercaptan formation during a period of exposure to the air. Working with smaller quantities of material than were employed by Nencki, I have never been able to obtain more than a trace of mercaptan (probably the methyl compound), and have usually been able to obtain no evidence whatever of its presence in fresh human material, whether from presumably normal persons or from such as were suffering from intestinal disturbance. But the failure to find mercaptan in the contents of the lower bowel does not prove that this substance has not been formed, for it is possible and even likely that there is some mercaptan production in higher portions of the large intestine, where it is certain that the gas would be readily absorbed.

As it appeared that something of interest might be learned from the study of the ability of fecal bacteria from various sources to form mercaptan when growing on a medium which does not readily yield this compound, a considerable number of experiments were made with suspensions of the mixed fecal bacteria from different persons. After growing the bacteria in a two per cent. peptone solution for twenty-four hours at the temperature of

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37°C., the entire culture (100 c. c.) was in each case transferred to a flask communicating through a calcium chloride tube with an Erlenmeyer flask containing isatin dissolved in concentrated sulphuric acid. A current of air was then drawn through both flasks so that any mercaptan given off from the culture flask would enter the isatin-sulphuric-acid flask. The presence of mercaptan is indicated under these conditions by a gradual change of the isatin solution from red to olive-green or grass-green.\(^1\) The method is not adapted for quantitative determinations but some idea can be gained through it of the quantity of mercaptan present in a culture, and it further serves to indicate differences in the amount formed in different cultures. Twenty-five milligrams of a one per cent. solution of methyl mercaptan suffice to gradually alter the red isatin solution (about 50 c. c.) to a deep green in the course of ten minutes. Reactions as strong as this are occasionally obtained from one hundred cubic centimeters of a bacterial culture, but quicker reactions of this intensity have not been found.

With the aid of the method\(^2\) here outlined, more than one hundred and thirty observations have now been made on aerobic cultures of mixed fecal bacteria.\(^3\) A fact which stands out clearly as a result of these observations is that the bacteria derived from normal persons (i. e., showing not more than moderate quantities of putrefactive derivatives in the urine and otherwise in good health) do not usually yield more than a trace of mercaptan when grown for twenty-four hours on a two per cent. peptone solution. In a number of instances, a pronounced trace of mercaptan has been obtained after prolonged aspiration through the apparatus mentioned above. In a few instances, a moderate reaction has been obtained after aspiration for ten or fifteen minutes, and such a reaction

\(^1\) This method has been used by Niemann, \textit{Arch. f. Hyg.}, xix, p. 126, 1893; also by Bauer, \textit{Zeitschr. f. physiol. Chem.}, xxxv, p. 346, 1902.

\(^2\) The method was in many instances supplemented by the use of the mercuric cyanide method.

\(^3\) The organisms were grown in 250 c. c. flasks and the volume of the peptone solution was 100 c. c. The upper portion of the culture was under aerobic conditions, the lower part, under anaerobic conditions.
has sometimes been repeatedly obtained from bacteria from the same person. Strong reactions were several times obtained from the organisms obtained from breast-fed babies apparently in good health, and once a strong mercaptan reaction was noted in the case of a growth made from material from a young man in good health but troubled with constipation. With these exceptions, I have noted strong mercaptan production only in cultures made from material derived from pathological sources. The strongest reactions were obtained from bacteria derived from persons suffering from pernicious anaemia, depressive mental states, infantile marasmus, fat diarrhoea, and cases of chronic intestinal indigestion (in children) characterized by abdominal distension, anaemia, and retarded development. In the cases referred to, mercaptan production has usually been a persistent rather than a transitory manifestation. In two cases of pernicious anaemia in which rapid improvement occurred in association with rest in bed and care in diet, the fecal bacteria ceased to produce mercaptan, coincidently with this improvement.

Thus the facts at present at our disposal indicate that the pronounced formation of methyl mercaptan by fecal bacteria growing on peptone solution is commonly a manifestation of pathological rather than normal bacterial activity, although it is doubtful whether its occasional production by micro-organisms from a human individual is to be regarded as necessarily unphysiological. Especially in the case of young children in good health is a moderate mercaptan a common occurrence. This conclusion is the more noteworthy because it is entirely different from the conclusion reached from a study of hydrogen sulphide production under similar condition. In the case of hydrogen sulphide formation, it may be said that this sulphur compound has almost regularly been obtained by growing fecal bacteria on a peptone medium, and that it is by no means un-common to find an abundant hydrogen sulphide production where no trace of mercaptan is obtainable.

We have as yet little accurate information as to the conditions under which methyl mercaptan is produced in the course

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1 The bacteria from certain acid stools from children may fail to make hydrogen sulphide, although under anaerobic conditions mercaptan may be produced.
of bacterial activity. Nencki regarded it as a result of anaerobic decomposition, but it appears that, although this view is for the most part correct, anaerobic conditions are not always essential to its formation. The bacteria from the feces of a patient with pernicious anemia grown on a peptone solution under aerobic conditions gave much hydrogen sulphide and no mercaptan; when the organisms were grown anaerobically (under carbon dioxide) there was an abundant production of mercaptan (presumably the methyl compound) in addition to hydrogen sulphide. On a medium containing asparagin, ammonium lactate, alanin, glycocoll, cystin, and salts, I obtained a very abundant production of hydrogen sulphide from fecal bacteria, but not a trace of mercaptan.

Pure cultures of the aerobic bacteria derived from feces capable of inducing mercaptan formation have failed to give this gas. B. coli communis growing on the peptone solution gives hydrogen sulphide but no mercaptan. B. putrificus I have not yet tried in pure culture, but Dr. Rettger tells me that it early produces mercaptan when grown on an egg-meat medium.

Although it is not at present clear what inferences may be safely made, from the formation of mercaptan by the fecal bacteria, with regard to mercaptan production and absorption in the intestine from which the bacteria were derived, it seems clear that further observations are desirable along lines suggested by the results embodied in this paper. It appears also desirable that a careful pharmacological study of the prolonged action of methyl mercaptan should be undertaken, and such a study is now under way in my laboratory.

Some observations which I have made upon bacteria found at early autopsies in various parts of the intestinal tract show that mercaptan-producing organisms may be present in the upper part of the ileum. Thus from an autopsy on a child of fifteen months, dead of pneumonia, the mercaptan production by the bacteria of the ileum was even more pronounced than that of bacteria obtained from lower levels. In the case of a child dead of marasmus, the stomach contained bacteria which made hydrogen sulphide abundantly and some mercaptan. The bacteria of the duodenum and jejunum made neither of these gases, but those of the ileum produced them both in abundance.
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