ON THE PRODUCTION OF FORMALDEHYDE BY INTESTINAL BACTERIA.

BY

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THE production of formaldehyde by bacteria when grown on suitable media has a significance of very considerable importance both from a bacteriological and from a pathological point of view, and, indeed, this property may in the case of certain organisms normally present in the intestinal canal have a direct influence on orderly metabolism.

Formaldehyde is an extremely toxic substance and, in common with all aldehydes, a powerful reducing agent; of its various tests Schryver's is regarded as the most delicate and reliable, and is carried out as follows: To 10 c.cm. of the suspected solution, 2 c.cm. of a 1 per cent. solution of phenylhydrazine hydrochloride are added, then 1 c.cm. of a 5 per cent. solution of potassium ferricyanide, and lastly 2 c.cm. of concentrated hydrochloric acid, when a pink colour appears if formaldehyde is present.

The following table shows the results of this test applied to certain organisms on the sixth day of incubation at 37.5° C. on peptone alone, and in combination with lactose, glucose, mannite, and inulin.

	Peptone.	Lactose Peptone.	Glucose Peptone.	Mannite Peptone.	Inulin Peptone.
Coliform, Group I ...	+	+	±	-	+
„ Group II ...	+	+	±	+	+
„ Group III ...	+	++	+	++	+
„ Group IV ...	-	±	±	-	-
Morgan's bacillus I ...	+	+	++	++	+
<i>B. faecalis alcaligenes</i> ...	-	-	-	-	-
<i>B. proteus vulgaris</i> ...	+	+	-	+	+
<i>B. proteus anindol</i> ...	-	-	++	-	- gr.
<i>B. proteus mirabilis</i> ...	-	-	-	-	- sl. gr.
<i>B. proteus zenkeri</i> ...	-	-	+	-	-
<i>B. paracoli</i> ...	-	-	±	+++	-
<i>B. enteritidis</i> Gaertner ...	-	-	±	+++	-
<i>B. aertrycke</i> ...	-	-	++	-	-
<i>B. cloacae</i> ...	-	±	- gr.	-	-
<i>B. lactis aerogenes</i> ...	-	-	- sl. gr.	-	-
<i>B. paratyphosus</i> A ...	-	-	-	-	-
<i>B. paratyphosus</i> B ...	-	-	++	+++	-
<i>B. paratyphosus</i> C ...	-	-	+	+	-
<i>B. typhosus</i> ...	-	-	+	-	-
Schmitz's bacillus ...	+	+	+	+	+
<i>B. putrificus coli</i> ...	±	-	-	-	±
Shiga's bacillus ...	-	-	+++	-	-
<i>B. of Flexner</i> V ...	+	+	+	++	+
„ „ W ...	-	-	±	+	-
„ „ X ...	+	+	+++	+++	+
„ „ Y ...	+	+	++	++	+
„ „ Z ...	+	+	±	-	+

+ to +++ denotes increasing strengths of formaldehyde.

- denotes no formaldehyde.

gr. denotes green coloration.

The pink colour soon fades, but if a little hydrogen peroxide is added it is brought back and intensified. In the case of *B. cloacae* the addition of hydrogen peroxide in this manner produces a deep orange colour on lactose peptone, and in the case of *B. putrificus coli* a deep red colour on inulin peptone.

Using standard solutions of formaldehyde for comparison purposes, the amount of formaldehyde manufactured by the various organisms appeared to vary from about 0.0001 to

0.0015 per cent.; with certain batches of media the colour was distinctly deeper than with others, possibly owing to inequality of steaming in preparation.

As an aid to classification and identification of bacteria this reaction should be of considerable value—for example, a marked distinction will at once be noticed between Schmitz's bacillus and Shiga's bacillus, between paratyphoid A and the other members of the Salmonella group, and the mannite reaction distinguishes between *B. aertrycke* and *B. enteritidis* Gaertner—the inability of paratyphoid A to form formaldehyde probably explains the fact alluded to by W. J. Wilson,¹ that of the Salmonella group paratyphoid A alone lacks the power of reducing sulphites to sulphides. Considerable variation will be noticed in the Flexner group—W and Z, for example, differ from each other and very markedly from the others; and the bearing of formaldehyde production on pathogenicity is a matter of some interest, as in this group vitality appeared to be more or less proportional to the power of its members to form formaldehyde—for example, when grown on glucose peptone Flexner X survived the longest, followed by Y. In this connexion it is a matter of interest that while staphylococci are negative to the formaldehyde test, such members of the streptococcus group as I have examined are slightly positive. With reference to this question of pathogenicity, the feeble manner in which glucose is attacked by the coliform group, with formaldehyde formation, as compared with more pathogenic organisms, is rather significant.

It is, however, in the very large coliform and *proteus* groups that this reaction will be found most useful from the point of view of identification, as it gives information hitherto unknown—for example, certain organisms belonging to the same group as far as saccharose and dulcitol fermentation is concerned react differently. In the *proteus* group also there is very great variation; those isolated in this laboratory from the stools of various patients have differed very markedly from the type organisms of the *proteus* group kindly supplied to me by Dr. St. J. Brooks from the National Collection.

It will be noticed that of the type specimens of the various coliform groups tabulated Group III is a much stronger producer of formaldehyde than the others, while Group IV is practically unable to manufacture it; and another important point is that certain of the coliform and *proteus* type of organism are able to form formaldehyde from peptone alone without the presence of a carbohydrate, as also are the Flexners with the exception of W.

It is therefore evident that the amount of formaldehyde present in the alimentary canal will entirely depend on the types of coliform organisms existing in it—for example, if organisms similar to Group IV are mainly present the quantity of formaldehyde produced will be minute, but if they are of the Group III type a relatively large amount of this toxic substance may require to be dealt with. In a routine examination of the stools of patients in this hospital for members of the *proteus* group it has been very noticeable that they were especially prevalent in cases of what is clinically known as exhaustion psychosis, and that the various organisms of this type isolated were all formaldehyde producers.

Bacteria do not appear capable of producing formaldehyde when free oxygen is excluded, and the question arises, Is free oxygen present in the alimentary canal? As far as I can ascertain it is not known to be, but I am informed by Professor Wace Carlier that sufficient O₂ exists in the lymph circulating in the intestinal villi in which the epithelial cells are bathed, and by attacking these cells organisms might easily obtain all they require, also they are capable of supplying themselves with free oxygen from various foodstuffs which contain atoms of O₂ in their molecules. Certainly it would seem that there can be little lack of free oxygen when such an intensely aerobic organism as the spirillum of Asiatic cholera can flourish in the alimentary canal.

I have been unable to find any reference in literature to the power of formaldehyde formation by bacteria, but that it may be normally present in the alimentary canal in degrees varying with the conditions obtaining, the kind of

food taken, and the nature of the bacterial flora, must now be recognized, and the possible effects of this compound on cellular metabolism deserve consideration in view of its extreme toxicity and immense range of chemical interaction.

Moore² states: "It is only in the minutest traces, the upper limit being about 0.001 per cent., that formaldehyde, which is one of the most intense of protoplasmic poisons, can be borne by living cells"; and "plants subjected in presence of light to solutions containing 0.001 per cent. of formaldehyde do not form as much starch as in its absence." A point of much interest and importance is that when any organic substance of biological origin is exposed to the short wave-lengths of ultra-violet light formaldehyde is formed; and the same is true, though more slowly, when exposed to sunlight; this fact, taken in conjunction with the known lethal effects of ultra-violet rays and sunlight on micro-organisms, is very suggestive.

A circular issued by the Local Government Board in 1906 stated:³

"The Board are advised that the presence in milk of formaldehyde to an amount which is ascertained within three days of collecting the sample to exceed 1 part in 100,000 raises a strong presumption that the article has been rendered injurious to health."

This statement was based largely on animal feeding experiments. In milk the formaldehyde is mostly gone in three days, and it is always greatly reduced in forty-eight hours.

At the instance of the United States Department of Agriculture, Dr. Wiley investigated the effect of formaldehyde on digestion and health. A daily dose of 100 to 200 mg. was given in milk to twelve healthy young men.

"No marked symptoms were noticed during the first ten days, but then headache and pains in the stomach and intestines became general in many cases, producing cramps, and in a few cases nausea and vomiting; a burning sensation in the throat was reported in the majority of cases, and in four of the subjects a well marked itching rash appeared; the metabolic functions were disturbed in a notable way."³

Mathews states that formaldehyde unites with free amino groups to form water and methylene addition products, and

"these methylene substitution products go over readily into methyl amino derivatives. This substitution of methylene for the positive element hydrogen in the amino group reduces the basicity of the amino group so that the acid character of the carboxyl comes clearly into evidence. . . . This is an important reaction because such methyl substitution products, which are quite common in plants and animals, are probably produced in this way."⁴

The fact that adrenaline is a methylated amine and that the powerful poisons choline, neurine, and muscarine are methyl derivatives shows the great importance attaching to any evidence bearing on the production of formaldehyde in animal tissues.

"It has not been shown that formaldehyde is an intermediary product of metabolism, but in the decomposition of the carbohydrates it is not impossible that small quantities may arise and that this formaldehyde is the source of the methylation."⁴

All aldehydes are active reducing agents, taking up oxygen and forming acids, the aldehyde group being converted into what is known as the carboxyl group HOCO ; formaldehyde is thus converted into formic acid, HCOOH . Formic acid and the formates are also strong reducing agents owing to the presence of the aldehyde group, and the former represents the final stage before complete decomposition into CO_2 and water is reached. Methyl alcohol is formed by the action of nascent hydrogen on formaldehyde, and the latter is partially transformed into formic acid and methyl alcohol by dilute solutions of the alkali hydroxides. The fact that formic acid is present in perspiration, from which it can be obtained by distillation,⁵ is a matter of some interest. Now methyl alcohol combines directly with calcium chloride, forming the compound $\text{CaCl}_2 \cdot 4\text{CH}_3\text{O}$, and if a 0.002 per cent. solution of formaldehyde containing some milk of lime be incubated overnight at 37.5°C . the amount of formaldehyde present will be found to be reduced to about 0.0004 per cent. as compared with a control solution similarly treated without the lime. A similar solution containing magnesia instead of lime was found to retain the full percentage of formaldehyde. That is to say, calcium hydrate neutralizes about four-

fifths of the formaldehyde in twelve hours or so. Loew⁶ in 1885 prepared a synthetic sugar, formose, by means of formaldehyde and milk of lime, and later⁷ the fermentable sugar methose by the use of magnesia.

Again, calcium forms saccharates of varying solubility with dextrose, sucrose, and laevulose, the compound with the last named being very insoluble. Since laevulose exists in all sweet fruits and is produced together with an equal amount of dextrose by the action of ferments on sucrose, the influence of calcium in preventing formaldehyde formation by directly entering into combination with the various sugars may be considerable.

It will be noted that in Wiley's experiments already alluded to four subjects to whom formaldehyde was given daily developed, in addition to headache and disordered metabolism, a "well marked itching rash." This is of considerable interest in view of the well known action of calcium lactate or chloride in such cases, in angioneurotic oedema, and in certain forms of headache. Wright held that this beneficial action of calcium was connected with increased coagulability of the blood. Professor Wild,⁸ however, states that clinically the effect of calcium in skin disorder is not due to increased coagulability of the blood, that the soluble salts have no particular value, *mistura cretae* being as useful as calcium lactate.

Presumably, therefore, calcium may exercise a beneficial effect on metabolism by interfering with the formation or by neutralizing excess of formaldehyde resulting from bacterial activity, and bearing on this Ransom's⁹ discovery, that in glycosuria the greater the amount of sugar the larger the excretion of calcium, is of much interest in view of the fact that adrenaline excess causes hyperglycaemia, and therefore the more adrenaline thrown out the greater will be the loss of calcium. Now, adrenaline being a methylated amine, its excessive formation is very possibly brought about by abnormal production of formaldehyde in the intestines, and a vicious circle thereby set up, leading to increased disorder of metabolism.

The part played by the liver as regards destruction of formaldehyde is of very great importance. Richter¹⁰ states that it is capable of forming glycogen from formaldehyde; if this is so it is highly probable that its cells can only deal with minute amounts, since, as already referred to, plants do not form as much starch in presence of light when subjected to solutions containing 0.001 per cent. of formaldehyde as in its absence. However, should excessive amounts of formaldehyde reach the liver over a considerable period of time, cellular deterioration must undoubtedly ensue. It is noteworthy that in Wiley's cases symptoms did not become evident for ten days, and that the metabolism was disturbed in a notable manner. This would fit in with a gradual failure of the liver owing to cellular degeneration.

Gustafon¹¹ has shown that the respiratory activity of *Aspergillus niger* is decreased when subjected to the action of acetone or formaldehyde, and Hopkins¹² has isolated a substance to which he has given the name of "glutathione," a compound of cysteine and glutamic acid, which is essential to the oxidation mechanism of the living cell. We have already seen how formaldehyde, by effecting the substitution of the carboxyl group for hydrogen in amino compounds, may aid in causing acidosis and thereby cellular anoxaemia, since the most favourable concentration of hydrogen ions for the oxidation of cysteine is the same as that of protoplasm. Glutathione is a sulphur compound, and in the reduced or cysteine form is recognized by giving the same colour reaction as acetone and other reducing agents with Rothera's nitroprusside test; it is present in an intense degree in animal cells and to a lesser degree in plants. Now formaldehyde has a strong affinity for sulphur, especially in presence of hydrochloric acid, with formation of thioaldehydes, etc., and of all elements sulphur is the most easily split off from the proteins; it would appear, therefore, probable that formaldehyde might directly interfere with metabolism by uniting with the protein sulphur needed for cellular oxidation.

It has been ascertained that hydrogen cyanide, which is the nitrile of formic acid, when given to animals exercises its poisonous effects by uniting with the unoxidized sulphur in the cell which it causes to be detached.

Protein sulphur is also attacked by the coli-typhoid group of organisms¹³ in the alimentary canal with the production of hydrogen sulphide, which, when absorbed in quantities, dissolves erythrocytes and contributes to anaemia; "some cystine is absorbed as such, taurine is produced from cysteine, and a portion of the cysteine probably passes the liver and is picked up by the tissue cells."¹⁴ From Hopkins's feeding experiments it appears that animals are unable to make sufficient tryptophane, tyrosine, lysine, or cystine for their needs, and that these amino-acids must be present in their diet; but if owing to an intrinsic or acquired abnormal condition of the upper intestine excessive invasion of this viscus by coliform organisms takes place, and such powerful substances as formaldehyde and its derivatives with strong affinities for sulphur are present to an abnormal extent, a serious reduction in the amount of cysteine available for the tissue cells seems inevitable. It is possible also that formaldehyde in low dilution may have a deleterious effect locally on the intestinal mucosa if its action is kept up for a prolonged period. One has in mind, too, in this connexion arteriosclerotic changes with deposition of calcium.

There are, then, various ways in which formaldehyde arising from bacterial action may interfere with cellular respiration which, arguing from the constantly observed association of confusional mental states with profound destruction of erythrocytes in cases of malignant malaria, and certain other facts bearing on states of acidosis, is, as I have pointed out elsewhere,¹⁵ of the utmost importance to the maintenance of mental health.

As far as treatment is concerned, obviously an attempt to replace the offending organisms by an innocuous type is very desirable. This is, however, a matter of considerable difficulty. It is probable that certain conditions exist in some individuals which favour a certain type of organism, and although the latter may be by artificial means supplanted by another, such as the *B. acidophilus*, a very short time after discontinuing treatment—which, to be effective, is by no means a simple matter and decidedly unpleasant to the patient—the offending organism returns again as actively as ever. A more practical method of dealing with the condition would appear to be the regular administration of liquid paraffin combined with occasional doses of salts and treatment directed to restoring general tone to the system. It is possible that liquid paraffin would mechanically tend to prevent formaldehyde production. The use of calcium salts might also be borne in mind.

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THE TREATMENT OF COLDS IN TUBERCULOUS SUBJECTS.

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Colds in the tuberculous cannot be regarded lightly, and any remedy which will lessen their incidence is obviously of importance. Some time ago it was suggested to me that I should try the effect of a vaccine for colds (Parke, Davis) on certain selected cases of tuberculosis of the lung, suffering from constant colds, who were attending Margaret Street Hospital for Consumption as out-patients.

Before treatment every case was tested by the "multiple cutaneous tuberculin test" of Ellis. For the purposes of this paper, those who reacted to dilutions of 1 in 500 old tuberculin are referred to as "hypersensitive," to dilutions of 1 in 100 as "sensitive," and to dilutions of 1 in 10 as "subsensitive." In addition a skiagram of the chest was made, the sputum examined for tubercle bacilli, and the

blood pressure taken; every case was provided with a four-hourly temperature chart, and only afebrile cases were dealt with. In each case the initial dose of the cold vaccine was 0.1 c.cm., with rapid weekly increments till 1 c.cm. was reached.

The results were disappointing. Out of 28 cases treated 16 did well, but in 12 the colds became distinctly worse and the condition of the patient was certainly not improved. I then tried a detoxicated vaccine (Genatosan, Ltd.), commencing with a smaller dose, 0.01 c.cm., with gradually increasing doses. It was administered twice weekly, and some twelve doses were given in each case. The results were better, but even with the lessened dose 2 out of 6 cases had severe reactions. I am now using a sensitized vaccine, commencing with 0.01 c.cm. The results, in that no ill effects have been produced, are satisfactory, but I have not employed it sufficiently long to form a definite opinion as to whether its curative effect is better than that of the "vaccine for colds."

Being impressed with the poor results I obtained from the administration of the "vaccine for colds," I have made an attempt to ascertain the cause. It must be either (a) in the patient, or (b) in the vaccine and the dose used.

Under the first heading we may consider (1) the presence or absence of tubercle bacilli in the sputum; (2) the sensitiveness to tuberculin and the blood pressure.

Presence of Tubercle Bacilli in the Sputum.

This seems to be immaterial. Of 8 cases with tubercle bacilli in the sputum 4 did well and 4 badly.

Sensitiveness to Tuberculin and the Blood Pressure.

I have grouped the cases into two large classes: (a) those with a blood pressure of 125 and under, and (b) those with a blood pressure of 130 and over. I have also divided the cases into subgroups according to sensitiveness to tuberculin. I have also tabulated cases of tuberculosis treated with tuberculin, and have subdivided these into those who were and those who were not suffering from colds. The tuberculin-treated cases were all afebrile, and the sputum was free from tubercle bacilli. I have placed in the columns marked "good" cases in which the colds disappeared and in which there was obvious general improvement. The remainder I have placed in the columns marked "bad." The cases recorded are too few to justify percentages as anything more than a means of memorizing results, but they perhaps make the position clearer. I have recorded these percentages to the nearest whole numbers.

TABLE I.—Blood Pressure 125 and under.

	Colds Treated with Vaccine for Colds (11 cases).		Treated with Tuberculin.			
			Colds (17 cases).		No Colds (24 cases).	
	Good	Bad	Good	Bad	Good	Bad
Hypersensitive, 1 in 500 ...	1	2=66%	11	0=0%	11	0=0%
Sensitive, 1 in 100 ...	3	3=50%	4	0=0%	7	0=0%
Subsensitive, 1 in 10 ...	2	0=0%	2	0=0%	6	0=0%

TABLE II.—Blood Pressure 130 and over.

	Colds Treated with Vaccine for Colds (17 cases).		Treated with Tuberculin.			
			Colds (11 cases).		No Colds (19 cases).	
	Good	Bad	Good	Bad	Good	Bad
Hypersensitive, 1 in 500 ...	5	2=28%	3	1=25%	5	2=28%
Sensitive, 1 in 100 ...	4	5=55%	3	1=25%	4	1=20%
Subsensitive, 1 in 10 ...	1	0=0%	3	0=0%	6	1=14%

These figures, though meagre, are significant. They seem to show that sensitiveness to tuberculin, not blood pressure, is the important factor in treatment with vaccines for colds. In treatment with tuberculin the converse holds