debted to Professor Japp, F.R.S.) The formula is there given as $C_6H_3(OH)(SO_3H)COOH$; and its formation by the action of sulphuric anhydride on salicylic acid (Mendius, 'Ann. Chem. Pharm,' vol. 103, p. 45), or by heating salicylic acid with concentrated sulphuric acid (Remsen, *ibid.*, vol. 179, p. 107). It is said to be very stable, and to undergo no change on heating with nitric acid.

The specimens of salicyl-sulphonic acid which I have used in my experiments were obtained from Messrs. Davidson and Kay, Union Street, Aberdeen.

III. "The Influence of Oxygen on the Formation of Ptomaines." By William Hunter, M.D., F.R.S.E. Communicated by Professor M. Foster, Sec. R.S. Received March 11, 1891.

(Abstract.)

A special interest attaches to the *rôle* of oxygen in the life-history of bacteria. Very wide differences exist, however, between different groups in respect of its importance. To the great majority a free supply of oxygen is absolutely essential for their proper growth and development; to a small minority the converse applies, growth proceeding best in the absence of oxygen, if indeed it is not entirely prevented by its presence; while, lastly, in the case of an intermediate group it seems almost immaterial whether oxygen be present or not, growth proceeding apparently equally well in both conditions, provided that the supply of food be otherwise suitable.

Of these three groups of "obligate aërobic," "obligate anaërobic" and "facultative aërobic" bacteria, respectively, the last has perhaps the greatest interest for the pathologist, as it is to it that the great majority of pathogenic organisms belong.

The question is thus an interesting one, to what extent the pathogenic properties of this class of bacteria are related to the power they, apparently under necessity, possess of obtaining their supply of oxygen from the food constituents themselves when the supply in the air is cut off.

The present paper deals with the results of an investigation undertaken in this relation.

It was necessary that the class of bacteria selected for study should be one whose pathogenic properties were not constant, but subject to variations presumably connected with the character of their surroundings.

The bacteria of ordinary putrefaction possess in a special degree this qualification, their chemical products differing much in character and poisonous action under different, for the most part as yet unknown, conditions.

The method chosen by which to gauge the influence of oxygen on the pathogenic properties of bacteria was to estimate the quantity of alkaloidal bodies or "ptomaines" formed in the putrefactive process, according as oxygen (1) was freely admitted; (2) was present in moderate quantity; or (3) was withheld altogether.

For our knowledge of the ptomaines of putrefaction we are chiefly indebted to the researches of Brieger. In their order of formation as well as complexity, the most commonly met are *choline*, $C_5H_{15}NO_2$; *cadaverine*, $C_5H_{14}N_2$; *putrescine*, $C_4H_{12}N_2$; *trimethylamine*, $(CH_3)_3N$; *dimethylamine*, $(CH_3)_2NH$; and *methylamine*, $(CH_3)NH_2$.

The ptomaines most characteristic of the early stages of putrefaction are the *diamines*, which include, in addition to cadaverine (pentamethylenediamine) and putrescine (tetramethylenediamine), other two isomeric with the former, but of different, as yet unknown, constitution—*neuridine* ($C_5H_{14}N_2$) and *saprine* ($C_5H_{14}N_2$).

With the exception of choline, all these bodies are non-poisonous; and choline only produces symptoms when given in very large doses.

In this respect they differ from another group which possess markedly toxic properties, *e.g.*, *muscarine* ($C_5H_{15}NO_3$), an oxidised derivative of choline, and *neurine* ($C_5H_{13}NO$), also obtainable from choline artificially by warming with baryta water; as also two other bodies to which Brieger gave the name of mydatoxine ($C_6H_{13}NO_2$) and *mydine*, $C_8H_{11}NO$.

While the poisonous bases are oxidised, the harmless bases are non-oxidised, a circumstance which led Brieger to conclude that oxygen plays an important part in the formation of poisonous alkaloids, and that a free access of oxygen favours the formation of ptomaines generally.*

The observations now recorded supply data for judging how far these conclusions are correct.

Their chief result is to show that the formation of the ordinary putrefactive ptomaines is favoured by the entire absence of oxygen; the quantity formed under such circumstances being several times greater than when oxygen is admitted.

Method of Research.

The method employed for the isolation of the ptomaines was that of Brieger. Equal quantities of extract of meat, obtained by extracting lean meat with cold water, were allowed to putrefy, for periods ranging from 5 to 8 days, under the three following conditions:—

(1.) *Free Supply of Oxygen.*—The fluid was placed in a large glass cylinder, open at both ends, kept in continuous rotation round a horizontal axis. The direction of rotation was alternately from right

* "Weitere Untersuchungen über Ptomaine." Hirschwald. Berlin, 1885, p. 27.

to left and left to right; the fluid was thus kept in continual agitation, and uniformly distributed over the inner surface of the cylinder.

(2.) *Moderate Supply of Oxygen.*—The fluid was placed in a wide-mouthed jar, and stirred freely from time to time.

(3.) *Exclusion of Oxygen.*—The fluid was placed in a narrow-necked bottle which it nearly filled; excess of oxygen at the outset was driven out by a stream of hydrogen; the bottle was then tightly closed by an india-rubber stopper through which passed a glass tube suitably bent and opening externally under mercury.

In (2) and (3), the vessels were maintained at a uniform temperature of 32° by being suspended in a water tank. In most of the experiments, a certain quantity of extract of pancreas was added, to hasten putrefaction, with 10 grams of $CaCO_3$ to prevent the injurious action of the acids formed in the early stages of the process.

The conditions of the experiments varied somewhat in other respects, either as regards the quantities of material used, or its nature, or the manner of dealing with it. The experiments made, eight in number, thus divide themselves into three series, each made up of two or three different observations under the conditions above noted.

The attempts made in the earlier experiments to isolate the individual ptomaines in the form of their platinum or gold salts failed, owing to the small quantities present.

Attention was afterwards confined to the diamines, and accurate quantitative results were obtained by use of benzoyl chloride—a reagent which, as Udranzky and Baumann have shown, forms bulky and stable derivatives with all bodies of this nature.

Results.

The results of the observations show:—

(1.) That a free supply of oxygen prevents entirely the formation of ptomaines, the only base found under such circumstances—and that too from the very first—being ammonia.

(2.) With one exception, all the experiments agree in showing that, as judged by the relative quantities of diamines formed, the greatest formation of ptomaines takes place when oxygen is entirely excluded.

The differences between moderate supply of oxygen and entire absence in this respect ranged from 2 : 1 to as much as 27 : 1 in the observations made, the greatest formation always taking place where oxygen was excluded.

The one exception to this can be explained by a difference in the procedure, the effect of which was probably to destroy a large number

of the diamines in the observation in which oxygen was excluded. The relation in this instance was reversed, viz., 1 : 3·8, the largest quantity being obtained where oxygen was admitted in moderate quantity.

(3.) Observations were also made on the effect of lengthening the duration of the putrefactive process when oxygen was entirely excluded. The result showed that on the 13th day the diamines were reduced to one-fourth of the quantity present in a similar amount of fluid, exposed to the same conditions, on the 7th day.

(4.) In all cases the bulk of the benzoyl compound obtained was made up of cadaverine, its melting point varying according to purity from 127°·5 C. to 129° C. Putrescine was only present in traces.

(5.) The results of the observations on the quality of the bases present were not so definite. The most definite symptoms of poisoning were obtained in one instance from the injection of a fluid which had putrefied in the absence of oxygen. They included prostration, increased peristalsis, and diarrhœa, and on another occasion rise of temperature.

Conclusions.

Certain conclusions are drawn from the above data, partly of a *special*, partly of a *general*, character.

The results are interpreted as tending to support Pasteur's original views as to the relation of fermentation processes to absence of oxygen, as against those more recently advanced by Schützenberger, Nägeli, Buchner, and others. They show for the putrefactive bacteria at least that a free supply of oxygen prevents fermentation altogether, as judged alike by absence of aromatic products and ptomaines, and by presence of ammonia from the very first.

They also show that Brieger's view before mentioned as to the necessity of the oxygen for the formation of ptomaines must be considerably qualified, the results obtained being entirely opposed to such a view.

As regards the influence of oxygen on the *quality* of the ptomaines formed, the conclusion is drawn that the presence or absence of oxygen is not the chief factor in determining the formation of poisonous, as distinguished from harmless, ptomaines; and that other factors, such as duration of putrefactive process and nature of material, are likewise incapable of doing so.

Both the formation and the character of poisonous ptomaines must be referred to individual characters of the bacteria present, probably also to the influence of "mixed infection," rather than to the physical conditions under which they act, important as the present observations prove certain of the latter to be in modifying in a very material way the fermentative action of bacteria generally.