

addicts, most of whom had the type of disease found in southern Italy, where delta infection is endemic.

Our results indicate that even among Scandinavian drug addicts chronic hepatitis B virus infection, otherwise a benign condition, may progress to serious liver damage once delta infection supervenes.

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Synthesis of histamine by *Haemophilus influenzae*

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Abstract

Recent findings suggest that bacteria might contribute to histamine concentrations in the sputum of patients with infective lung disease. Ten isolates of *Haemophilus influenzae* from patients with acute exacerbation of chronic bronchitis and emphysema, together with two reference strains, were incubated at 37°C for 72 hours. Serial estimations of histamine concentrations by high pressure liquid chromatography showed significant increases at 24 and 48 hours; no increases were evident in the control samples. These findings suggest that *H influenzae* might contribute to inflammation and limited airflow in infective lung disease by producing histamine.

Introduction

We recently showed large quantities of histamine in the sputum of patients with chronic bronchitis and cystic fibrosis; concentrations increased substantially when samples were incubated at 37°C. This phenomenon was prevented both by heating samples at 100°C before incubation and by adding antibiotics, suggesting bacterial

synthesis of histamine.¹ As the experiments did not clearly indicate which species might be responsible we investigated the ability of organisms commonly isolated from sputum to synthesise histamine. We report here our findings for *Haemophilus influenzae*, a common respiratory pathogen.

Methods

The strains of *H influenzae* studied included 10 isolates from patients with acute exacerbation of chronic bronchitis and emphysema and reference strains NCTC 8467 Pittman type b and NCTC 7918 Pittman type f (now unencapsulated). The culture medium used was a modified trypticase soy broth supplemented with histidine (1g/l), pyridoxine (5 mg/l), and 5% Fildes extract (Oxoid Ltd, UK). Each strain was incubated in trypticase soy broth at 37°C overnight, and 1 ml of this culture was then added to 20 ml trypticase soy broth in a sterile polypropylene container, which was incubated at 37°C for 48 hours with gentle agitation. Samples taken for histamine analysis were stored at -20°C. Histamine was analysed by high performance liquid chromatography.²

Duplicate cultures of the 12 strains were prepared as above. Histamine concentration was estimated at 0 and 48 hours and purity plates inoculated to check for contamination. Bacteria were counted at 48 hours. Two broths that had not been inoculated were included as negative controls. To check reproducibility four replicate cultures of two isolates found to produce histamine were set up and samples taken at 0, 24, and 48 hours. Duplicate cultures of one strain that produced histamine were also set up as above and samples taken at 0, two, four, six, nine, 12, 24, 31, and 48 hours for histamine analysis and bacterial counts.

Results

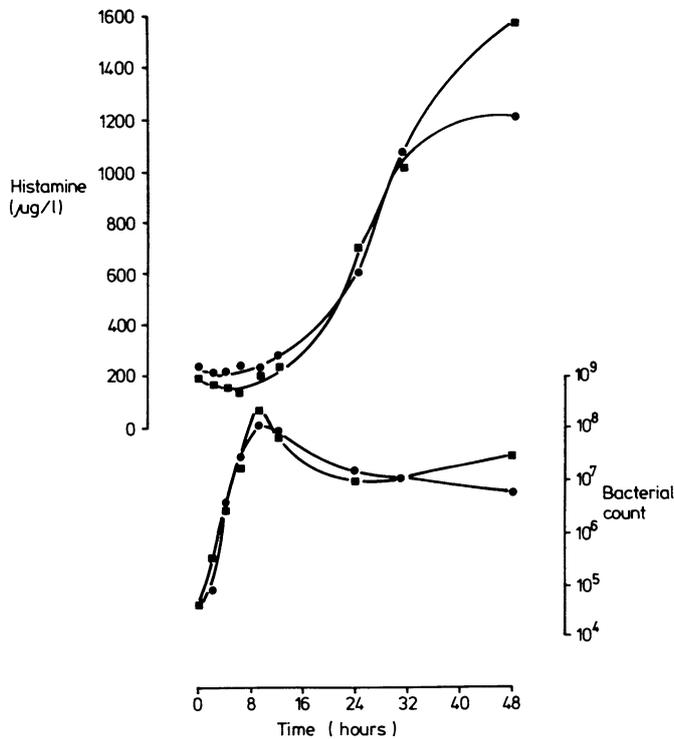
Seven of the 12 isolates produced histamine, the concentration in the culture medium increasing in the range 127-3360%. All replicates showed highly significant increases in histamine concentrations at 24 and 48 hours ($p < 0.01$ and $p < 0.001$ respectively). Controls showed no increases. The figure shows the relation between bacterial growth and production of

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Relation between histamine concentration in culture medium and bacterial growth in duplicate cultures.

histamine for one isolate in duplicate cultures: histamine is produced during the stationary phase. High pressure liquid chromatography mobile phase adjustment, treatment with diamine oxidase, and thin layer chromatography all confirmed the presence of histamine in cultures. Extracts of cultures induced contraction in preparations of isolated guinea pig ileum, a reaction that showed dose related antagonism by mepyramine.

Discussion

Histamine concentrations in sputum in infective lung disease are high and comparable with those found in asthma, though mast cells seem a less likely source. Synthesis of histamine has previously been shown in a variety of bacterial species, mainly Gram negative organisms. Indeed, high histamine concentrations in the tissues of fish probably account for the symptoms and signs associated with the ingestion of spoiled *Scombridae* and *Scombridae* such as tuna and mackerel and possibly other species. Bacteria that have been implicated include *Proteus* spp, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Clostridium perfringens*, and some species of *hafnia*, vibrio, providencia, citrobacter, and lactobacillus.^{3,4} This is the first report of synthesis of histamine by *H influenzae*.

Effects of histamine on the lung include constriction of smooth muscle, stimulation of vagal afferent nerve endings and mucous glands, and increased permeability of bronchial epithelium.⁵ Our results suggest that *H influenzae* might contribute to inflammation and limited airflow in infective lung disease by producing this highly potent biogenic amine.

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100 YEARS AGO

A CANDIDATE FOR A DRUNKARD'S RETREAT.

Under this heading, a daily contemporary has reported an application by two gentlemen, on behalf of a third person, for admission into a "drunkard's retreat." The applicant was described as "rather strange in his manner," and was cross-examined by the magistrate as to his knowledge of the provisions of the Acts under which he wished to be detained.

The Acts in question were only passed after strenuous efforts; and the difficulties inherent to these and similar enactments were only overcome by much devotedness on the part of those philanthropic persons who have made the treatment and cure of the inebriate the object and purpose of their life. It might have been hoped, therefore, that so much self-abnegation and goodwill would meet with due appreciation and assistance at the hands of those who, in their official capacity, are called upon to intervene in the case, as well as by the general public. We cannot say that these hopes and anticipations have been altogether realised. From time to time a crotchety magistrate has prolonged a painful situation by unnecessary quibbles; while the police authorities, who are perhaps naturally disposed to look upon "running in" as a sovereign remedy for inebriety, do not always show that alacrity in acting up to their instructions which might reasonably be expected. These little hindrances, however, hamper rather than prevent due recourse from being had to these homes; but such cannot be said of the practice for which a precedent has now been established. If the unhappy victim of a degrading vice is to be labelled forthwith as an "applicant for admission to a drunkard's retreat," his name and his shame blazoned forth in all the daily papers, with a reversionary interest in a heritage of shame, while he is cross-examined in open court as to his acquaintance with the details of the law under which he consents to alienate his liberty for a time, then we may be sure that the number of applications will be small.

It is already difficult to bring the repentant sinner to make and indict a

formal confession of his failing; and if the publicity, which is already unjustifiable in ordinary police reports, is, in future, to be accorded to these particular cases, no one, with enough self-respect left to desire reformation will be induced to face the unutterable shame of such a declaration.

We would, in the first instance, appeal to the Press generally to decline such contributions; and, should this fail, it would not be amiss to take measures for the introduction of a Bill which shall deal with the question of publicity—at any rate, as far as names are concerned—of affairs of simple police. The very least that can be done will be to cause such applications to be made *in camera*; and, possibly, means may ultimately be found whereby the intervention of so public a functionary as a magistrate may be avoided.

Justice and mercy suggest the propriety of throwing a veil over the frailties of our fellows, especially when the very effort indicates a desire to regain self-command, and avoid the inevitable consequences of a degrading vice.

The reports of these homes are, on the whole, encouraging. Even where cure has not been effected, the good is beyond and above suspicion. Every march stolen on so redoubtable an enemy as habitual drunkenness, is an achievement of which the temperance church militant may justly be proud; and will serve to encourage further efforts in the same direction. Of course where inebriety is only the expression of what we may term a "neurotic diathesis," the good can only be temporary, and the benefit from treatment in such cases is not to be measured by the permanency of its results; but many—and, doubtless, the vast majority—of cases of chronic inebriety, are attributable simply to the besotting influence of pernicious social customs—customs which we are glad to see surely, if slowly, becoming less general. In this class, the work may be undertaken with fair hope of lasting success, and the advocates of this method of temporary restraint may legitimately call on their friends to "rejoice over one sinner that repenteth." (*British Medical Journal* 1886;ii:28.)

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