Toadstools and Mushrooms as a Source of Antibacterial Substances Active Against *Mycobacterium phlei* and *Bact. typhosum*

In a survey of more than two hundred kinds of mushroom and toadstool collected in South Australia, antibacterial activity was detected in several members of *Cortinarius* and in one member of *Psalliota*. The tests were made by the cylinder plate method of Heatley[1], with watery extracts obtained by grinding up the mushroom or toadstool in a mortar with a little water. All the active extracts inhibited the growth of *Staph. aureus*, but extracts of only two fungi showed wider activity. *Cortinarius rotundisporus* and *Psalliota xanthoderma* produced extracts which inhibited not only the Gram-positive organism, *Staph. aureus*, but also the Gram-negative organism, *Bact. typhosum*, and the acid-fast organism, *Myco. phlei*.

The crude extracts from *Cortinarius rotundisporus* showed greater activity against the staphylococcus than against *Bact. typhosum*; but the extracts from *Psalliota xanthoderma* showed approximately equal activity against these two bacteria. Against *Myco. phlei* the activity of all extracts was less than that against *Staph. aureus*, but nevertheless was well marked.

It is probable that the substance responsible for the antibacterial activity is different in each fungus, as the crude extracts of *Psalliota* were more readily inactivated by heat and alkalinity than were the crude extracts of *Cortinarius*. Further work on these antibacterial substances is proceeding and will be reported elsewhere.

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Inhibition of *Mycobacterium tuberculosis* by Garlic Extract

Of late there has been an intense search for antibacterial substances effective against acid-fast bacteria, particularly *Mycobacterium tuberculosis*. Apart from the chemotherapeutic work on sulphones and related compounds, a number of antibiotics of fungal origin have been reported[1–7]. However, antibacterials from the higher plants do not appear to have been tested against *Mycobacterium tuberculosis*.

Garlic is one of the well-known remedies for tuberculosis, according to the indigenous Ayurvedic and Unani systems of medicine. In continuation of our work on the chemistry of garlic[8–10], we prepared an antibiotic extract from *Allium sativum*, Linn., by a slight modification of the method of Cavallito and co-workers[11], who found it to be effective against Gram-positive and Gram-negative organisms. However, they did not extend their work to acid-fast bacteria.

In our experiments, *Mycobacterium tuberculosis* (human strain *B* 52, H_1, Kasauli) was employed by us as test organism for *in vitro* and *in vivo* studies. In the first group of experiments, flasks containing Long's medium (with ammonium malate instead of asparagine) with the extract in the form of emulsion so as to give concentrations of 2 mgm., 6 mgm., 12 mgm. and 18 mgm. per 100 c.c. of the media were inoculated with two loopfuls of *Mycobacterium tuberculosis* from a slant culture and incubated. No growth was observed after three weeks in any of the flasks containing the extract, while in the control the bacillus grew profusely. In the flasks containing 2 mgm. garlic extract per c.c., slight curdy submerged growth was observed from the fourth week onwards; but it was very slight and slow. This was confirmed by repeated experiments.

We then inoculated 17 c.c. of Long's malate media containing 6·5 mgm. of extract per c.c. in the form of emulsion with three loopfuls of the bacillus from a slant culture and incubated it for 24 hours with a control, similarly prepared but without the extract. Subcultures were made from the two samples on Dorset's egg media, and 2 c.c. portions were injected subcutaneously in the groin region into guinea pigs. After four weeks, no growth was observed in the subcultures from the tube containing garlic extract, while there was good growth in sub-cultures from the control tube. The animals are under observation at present.

In the light of the above observations, garlic extract *in vitro* is bacteriostatic in low concentration and probably bactericidal in higher concentrations. Experiments are now in progress to test the efficacy of the extract in guinea pigs immediately after infection.

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Bronchial Asthma Caused by the Trickling Sewage Filter Fly (*Psychoda*): Inhalant Insect Allergy

Allergic sensitivity to the emanations of various insects (mayfly, caddis fly, moths and butterflies, etc.) have been reported. The trickling sewage filter fly (*Psychoda* spp.) can now be added to the list of such insects causing inhalant bronchial asthma.

Thirteen cases (two Europeans and eleven Africans) of bronchial asthma of varying degrees of severity were investigated at a sewage works in the Transvaal. In all these, the condition was characteristically present in the warmer months of the year and the symptoms occurred during work in the vicinity of the sewage trickling-filter beds where masses of *Psychoda alternata*, Say. accumulate in the shady parts of the grounds and in the neighbouring buildings. The African patients, in addition, developed attacks at night in their nearby compounds, which were heavily infested with the flies. All the subjects claimed to be symptom-free when on vacation or away from the sewage works.

The *Psychoda alternata* is present in great abundance in many sewage works of the Transvaal where the trickling-filter is used for sewage purification. They are plentiful throughout the summer until May, when the first frosts appear. At that time *Psychoda severini*, Tonn., becomes more prominent, although occurring in relatively small numbers, which rapidly diminish with the approach of the warm weather in September.

In view of the striking correlation of the asthmatic symptoms with contact with the *Psychoda*, allergic investigations were carried out on the employees.

The first European case seen was especially closely studied. He had no family history of allergy and, by skin tests, was not found sensitive to any of the commoner inhalant substances (animal dander, feathers, dusts, etc.) nor to a large variety of South African pollens. He gave strongly positive skin reactions, however, to extracts prepared from the *Psychoda*. Passive transfer tests (Prausnitz–Kustner) with the patient's serum were positive. Skin tests gave negative results with extracts of *Psychoda* larvæ and with the hairs of the fly obtained as siftings by gentle agitation of the *Psychoda* in a fine sieve.

The inhalant allergen in *Psychoda* sensitivity was demonstrated to be the dust resulting from the disintegration of the bodies of the flies, which readily become friable.

When the insect origin of the patient's condition had been established, a survey was made of the 35 African employees on the sewage works. Eleven of these who were found to be sufferers from asthma were submitted to skin tests with *Psychoda* extracts. Five of the patients gave a strongly positive reaction and, in four instances where the passive transfer test could be carried out, the result was positive in two of the cases. In a control group of twelve non-asthmatic African employees, no significant reactions were obtained with the *Psychoda* extracts.

It is suggested that all allergic respiratory conditions occurring at, or in the neighbourhood of, sewage works should be especially investigated from the point of view of *Psychoda* sensitivity. Apart from the prevention of the fly nuisance, the importance of the control of *Psychoda* breeding in filter beds assumes a new significance.

Desensitization of the European case with *Psychoda* extract has already been commenced, and a survey of other sewage works has been arranged in order to determine the prevalence of allergic conditions due to *Psychoda*.

Details of the cases, the conditions obtaining at local sewage works and a summary of the methods used in the control of *Psychoda* are being published elsewhere.

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Alloxan Diabetes in a Ruminant

In the ruminant, the digestion of cellulose and other related carbohydrates is achieved through the conversion by the symbiotic flora of the paunch to simple fatty acids with the evolution of methane and carbon dioxide. If the fatty acids which are absorbed constitute the animal's main source of energy, it is possible that ruminants have a novel intermediary energy metabolism[1] in which sugar may play a subordinate part. It is well known that the concentration of glucose in the blood of sheep and goats is normally much lower than that of animals which do not ruminate. Furthermore, relatively large quantities of insulin are necessary to reduce the blood sugar concentration of ruminants to the low levels which lead to hypoglycæmic distress[2,3]. Man and the usual experimental animals go into convulsions in such circumstances; sheep do not go into convulsions, they subside into an apathetic condition followed by coma.

The discovery that the intravenous injection of alloxan will bring about a specific necrosis of the β cells of the islet tissue of the pancreas[4], and result in a diabetic condition in the rabbit[5] and in other animals[6], led to the investigation of sheep when subjected to similar treatment.

Experimental studies with six Merino and six Merino-Corriedale ewes indicated that the intravenous injection of 90 mgm. of alloxan per kilo body weight produced typical diabetes mellitus. Marked individual idiosyncrasy, however, was observed: whereas serious liver and kidney damage supervened on the diabetic condition in some of the animals, two were rendered diabetic without extra-pancreatic complications and were maintained in this condition for 47 and 52 days respectively, when they were slaughtered for autopsy.

Study of the glucose concentration in the blood and total ketone and glucose excretion provided ample evidence of the immediate triphasic response and subsequent hyperglycæmia, with glycosuria and ketonuria typical of alloxan diabetes. A sustained hyperglycæmia of 140–200 mgm. glucose per 100 ml. of blood, with an average daily excretion of about 70 gm. of glucose and 10 gm. of total ketone bodies, was observed. The diabetic syndrome could be controlled with insulin injections.