in vitro tests with human-type tubercle bacillus, Prof. W. H. Tytler, Welsh National School of Medicine, Cardiff, to whom our thanks are due, stated that there was a fairly sharp end-point showing practically complete inhibition of growth at 1 : 50,000–1 : 100,000; the inhibition was apparently permanent (towards other organisms the effect was a bacteriostatic one in low concentrations, but bactericidal in stronger solution) and was not affected by at least moderate concentrations of serum. This grade of activity is comparable to the most powerful of various experimental drugs hitherto known which could possibly be used in human treatment. In further tests kindly carried out by Dr. A. T. Fuller, National Institute for Medical Research, London, the pigment was active at *c.* 1 : 100,000 against a hæmolytic *Streptococcus* and active also against *Clostridium welchii*. In mouse tests a dose of 10 mgm. was tolerated, so that the pigment seems relatively non-toxic.

Details of this work will be published elsewhere; meanwhile fuller chemical and biological examination of the materials mentioned above and the similar ones from other Fusaria is being carried out, as well as appropriate synthetic work.

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Biologically Active Metabolic Products of the Mould *Metarrhizium glutinosum* S. Pope

In 1944 we obtained a culture of a *Metarrhizium* sp. from the United States Department of Agriculture, which had been described by Greathouse, Klemme and Barker[1] as an active decomposer of cellulose, particularly suitable for *in vitro* assessment of textile preservative treatments. This fungus was afterwards described by Pope[2] as a new species, *M. glutinosum*. Our organism agrees with Pope's description. We have found that this mould, when grown on a variety of synthetic media, produces fungistatic culture filtrates. From cultures of this mould on Raulin Thom medium, supplemented by 0·01 per cent 'Difco' yeast extract, we have isolated a substance for which we propose the name 'glutinosin', in yields of the order of 15 mgm. per litre of culture filtrate.

Glutinosin can be isolated from active culture filtrates by extraction with ether, *n*-butyl alcohol or petroleum ether. We have found the latter solvent preferable. By evaporation to dryness and crystallization from ethyl alcohol, glutinosin is obtained as thin, colourless plates. These crystals do not melt at 300° C. and they only darken slowly at this temperature; they contain no sulphur, nitrogen or halogens and give no ash on combustion. Analyses are given in Table 1. Glutinosin is optically active; for a 0·2 per cent solution in benzene, $[\alpha]_D^{20}$ is approximately + 54°.

Table 1.

	I	II	$C_{46}H_{66}O_{17}$ requires
C%	64·7	65·2	64·6
H%	6·7	7·0	6·8
Molecular weight (Rast)	920	755	892·7

Glutinosin is somewhat specific in its antifungal activity. In Table 2 is shown the minimum concentration needed to inhibit completely germination of spores of a number of fungi, at *p*H 3·5. The variation in the effect of glutinosin on different species is noticeable. Glutinosin is not markedly antibacterial; growth of *St phylococcus aureus*, *Salmonella typhi* and *Escherichia coli* is not inhibited by concentrations of 100 μgm./ml. Aqueous solutions of glutinosin are relatively stable. At 25° C. aqueous solutions maintained their fungistatic activity unchanged for 10 days in a *p*H range of 2·9–7·6; at *p*H 8·4 there were indications of slight loss of activity. Thus, like viridin[3], glutinosin is a specifically antifungal antibiotic, but is much more stable.

Table 2.

Fungus species	Least concentration (μgm./ml.) of glutinosin preventing spore germination
Botrytis allii Munn	0·2
Penicillium expansum Thom	25·0
P. digitatum Sacc.	1·0
Fusarium cæruleum (Lib.) Sacc.	0·8
F. graminearum Schwabe	>50·0

Glutinosin is not the only biologically active material produced by *M. glutinosum*. After handling the first large-scale cultures of this mould, one of us suffered from severe facial inflammation, followed by desquamation of the skin. There was considerable local irritation; the eyelids were particularly painful. We have since established the fact that, if a little culture filtrate is applied to the upper arm, a typical inflammation appears in forty-eight hours. All of six subjects tested were sensitive, though in varying degrees. The symptoms produced are similar to those described for the dermatitis caused by contact with poison ivy (*Rhus toxicodendron*). The substance responsible for this effect is extracted along with glutinosin by the methods described above, and remains in the alcoholic mother liquors after the glutinosin has crystallized out. Glutinosin has no irritant properties. So far as we are aware, this is the first record of the production by a mould of a substance with these properties. We have found it necessary to make liberal use of barrier creams when working with cultures and culture extracts of this mould.

Work on the chemical and biological properties of these substances is being continued, and our results will be reported in greater detail elsewhere.

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Antibiotic Material from *Bacillus licheniformis* (Weigmann, *emend.* Gibson) Active Against Species of Mycobacteria

About a year ago, one of us (P. D'A. H.) made the observation that sterile filtrates from cultures of a stock strain of a micro-organism, believed to be *Bacillus subtilis*, exerted an inhibitory action on the growth of *Mycobacterium phlei*. The organism has now been identified as *Bacillus licheniformis* (Weigmann, *emend.* Gibson) and a considerable advance has been made in the concentration of an active material, which is found to be inhibitory *in vitro* also against *M. tuberculosis*. A preliminary account of our results is now communicated because of reports which have appeared recently of the presence of antibiotic material in cultures of *B. subtilis*. One of these reports[1] first came to our notice, and the others[2–3] were published, while our own investigation was in progress. It is possible that some of these investigations, as well as earlier ones[4–12], may deal with the same species as we have used, since *B. licheniformis* is not always distinguished from the closely related *B. subtilis* and the distinction has not, in fact, been recognized universally[13]. Identity of our chief fraction of active material with either 'subtilin'[1] or 'bacitracin'[2] does not seem likely, for certain properties of subtilin do not agree with those of our material—notably the solubility of the former in aqueous ethanol—and the method of extraction of bacitracin is not applicable to our material.

B. licheniformis is grown on an autoclaved synthetic medium containing in one litre potassium monohydrogen phosphate 1 gm., sodium citrate 0·5 gm., magnesium sulphate ($MgSO_4.7H_2O$) 1 gm., asparagine 5 gm., glucose 30 gm., iron ammonium citrate 17 mgm., manganese sulphate 3 mgm., distributed in shallow layers in flasks. Solutions of the last three components are sterilized separately before mixing. Inoculation of the medium by *B. licheniformis* is carried out by adding to it 2 per cent of its volume of a culture grown in half-filled test tubes of a similar medium. Sucrose appears to give a lower yield of antibiotic activity than glucose; glycerol gives good growth but negligible activity. The cultures are kept at 37° and are harvested in from six to twelve days.

It was found early in the investigation that filtrates obtained by passage through Seitz-type filters had little activity in comparison with the product of autoclaving the culture at a *p*H between 2 and 3. This led directly to the conclusions that the active material was thermostable and, moreover, was bound to the bacterial bodies and was brought into solution by heating with very dilute acid. Autoclaving was adopted as the routine method of sterilizing for test purposes, particularly as there was evidence that the filters used adsorbed the active material.

Two methods have been employed for the first stage of the extraction. Either the culture is acidified to *p*H 2·5 and treated with 3 volumes of 95 per cent ethanol and the coagulum is collected and extracted by boiling with 0·5 volume of 0·4 per cent acetic acid for 45 minutes, or the culture is brought to *p*H 2·5 and autoclaved at 10 lb./in.² steam pressure for 10 min. The supernatant solution obtained after centrifugation of the product in either case is treated with 4 volumes of 95 per cent ethanol and the precipitate is collected. Small amounts of an inactive picrate have been obtained from this material and active material recovered by precipitation of the supernatant solution with ethanol. The white solid thus obtained, in a yield of 0·9–1·8 gm. from 1 litre of culture, is soluble in water, giving viscous solutions. It gives positive Molisch and Sakaguchi tests. Active material dialysed slowly through a cellulose membrane.

The biological activity of this crude concentrate has been more fully investigated than that of the more highly purified extracts, free from polysaccharide, which have now been obtained. Solutions in water are prepared for test by sterilization at *p*H 2–3 for 10 minutes under 10 lb./in.² steam pressure and serial dilutions are made in broth. 5 ml. of broth are inoculated on the surface with *M. phlei* and growth is observed after four days at 37°. The highest dilution at which inhibition is complete is observed, and the reciprocal of this figure is taken as the number of units in 1 ml. of the test solution. Cultures containing 320–1,280 units per ml. have yielded crude concentrates containing 100,000–300,000 units per gm. of solid, and, in general, about 50 per cent of the original activity is recovered. Crude concentrates having activity of this order against *M. phlei* inhibited the growth of *Staph. aureus* at 1 : 80,000, and of *B. licheniformis* at 1 : 10,000, when incubated in broth for 18 hours at 37°; *E. coli* was not inhibited at 1 : 5,000. In broth containing 50 per cent of horse serum, growth of *Staph. aureus* was inhibited at 1 : 40,000. Inhibition of growth of a virulent human type of *M. tuberculosis* grown for three weeks in deep culture in a modified Kirchner medium occurred at a dilution of 1 : 20,000. The crude concentrate was tolerated by mice