LETTERS TO THE EDITORS

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Moulds Producing Penicillin-like Antibiotics

IT has already been found that penicillin-like antibiotics are produced by a number of moulds besides *Penicillium notatum*, including species of both *Penicillium* and *Aspergillus*[1–15].

Penicillin-like antibiotics have now been shown to be produced by a further five species of *Penicillium*:

P. steckii Zal. National Collection of Type Cultures No. 3950. The observation that this mould produced an inhibitor active against *Staph. aureus* but not against *Bact. coli* was reported by Wilkins and Harris[16].

P. chloroleucon Biourge.

P. asperulum Bain.

P. crateriforme Gilman and Abbott (Daltilo-Rubbo's strain).

P. griseo-fulvum Dierckx.

The last four strains were kindly supplied by Prof. J. Westerdijk, of the Centraalbureau voor Schimmelcultures, Baarn.

In each case the antibacterial activity developed on a number of media, of which the best was modified Czapek-Dox with lactose substituted for glucose, and with the addition of corn-steep liquor.

The antibiotics produced on this medium all had the following properties: active against *Staph. aureus*, not against *Bact. coli*; extracted into organic solvents at *p*H 2 and re-extracted into water at *p*H 7; inactivated by acid and alkali; slowly inactivated by heating at *p*H 7; completely inactivated by penicillinase and by copper ions. The combination of these properties is satisfactory evidence of the penicillin-like nature of the active substance. The antibiotic from *P. chloroleucon* was completely inactivated by methyl alcohol, but with the other four antibiotics the inactivation by methyl alcohol was only partial.

P. steckii, *P. chloroleucon* and *P. asperulum* bear some resemblance morphologically to the *P. chrysogenum-notatum* group; the other two species are widely separated both from this group and from each other.

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Effect of Calcium on the Production of Botulinus *D* Toxin

SOME time ago, Dr. K. F. Meyer, director of the Williams Hooper Foundation, told us that he had obtained high yields of botulinus *A* and *B* toxins on media containing filtered alkalinized corn steep liquor. Lamanna, McElroy and Eklund[1] have since reported similar results.

We were unable to obtain high yields of *D* toxin on corn steep media until we added back to the media the ashed precipitate from the alkalinized corn steep filtrate. Eventually we found that the ash could be replaced by calcium, in the form of the chloride, lactate, phosphate, carbonate, etc. The optimum amount of soluble calcium was about 30 mgm. per cent. The media contained 0·5–1·5 mgm. per cent before the addition of calcium. The addition of lactic acid or calcium lactate to media containing excess calcium carbonate resulted in further improvement. The medium now used is prepared as follows. Unconcentrated corn steep liquor is precipitated at *p*H 8·5–9·0. The precipitate is removed and the liquor diluted with an equal amount of water. Thirty mgm. per cent calcium is added as lactate, together with 0·5 per cent calcium carbonate. The *p*H is adjusted to 7·2, and the medium sterilized at 15 lb. pressure.

An interesting point was the relatively large amount of iron required for adequate toxin production. The optimum was about 6 mgm. per cent, but amounts as high as 60 mgm. per cent did not lower toxin yields. Iron, magnesium and phosphorus were, however, present in sufficient amounts in the steep, and the major deficiency was calcium.

A typical result is shown in the accompanying table.

Corn steep medium precipitated at *p*H	Calcium added	Value of toxin	
		No. M.L.D. per c.c. for mice	* Prov. units antitoxin bound by 1 c.c.
7·5	Nil	No growth	No growth
8·5	Nil	No growth	No growth
9·0	Nil	No growth	No growth
9·0	$CaCl_2$	$>10^6$	500
9·0	$CaHPO_4$	$>10^6$	250
9·0	$CaCO_3$	$>10^6$	500
9·0	$Ca(C_3H_5O_3)_2$	$>10^6$	1000

* Prov. = provisional laboratory units.

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Poly-agglutinable Red Cells

IN a recent communication in *Nature*, Basil-Jones, Sanger and Walsh[1] described a case of puerperal pyæmia the red cells of which (Group *O*) were agglutinated at 20° C. but not at 37° C. by 45 out of 45 normal sera of all groups. The peculiar behaviour of these red cells appeared to be similar to that described by Levine and Katzin[2] and Gaffney and Sachs[3]. The latter authors described their phenomenon as 'poly-agglutinability' of red cells.

In 1943 we observed two cases which were similar to those eventually published by Gaffney and Sachs.

Case 1. ♀ aet. 29, Group *O*—incomplete abortion. Fresh, washed red cells were agglutinated at room temperature but not at 37° C. by 38/39 sera, selected from all four *ABO* groups. The degree of agglutination was variable. Repeated washing of the red cells with ice-cold saline and saline at 45° C. did not affect the agglutinability. The patient's serum contained α and β *iso*-agglutinins active at room temperature and 37° C. Non-specific cold agglutinins active against the patient's cells and group *O* cells though present at 5° C. were only very weak. The patient's red cells hæmolysed with tap water did not transmit the poly-agglutinability to other normal group *O* cell suspensions. They thus differed from red cells showing the Hübener–Thomsen–Friedenreich phenomenon.

The agglutinating property of normal sera for the patient's red cells could be removed by absorption at 5° C. with equal volumes of the patient's washed red cells. Recovery in saline at 37° C. of the agglutinin was also carried out from the cold-washed cells used for the absorption. The recovered agglutinin agglutinated at room temperature the patient's red cells but not normal red cells of groups *O*, *A*, *B* and *AB*. Therefore, though a cold agglutinin, it appeared to be specific for the patient's cells, and not the non-specific cold agglutinin.

Case 2. ♀ aet. 37, Group *O*—hysterectomy for parametritis. Qualitatively all the findings in Case 1 applied to Case 2. She was met six weeks after Case 1 and a further sample of Case 1 was obtained for comparison. By this time the red cells of Case 1 were agglutinated at room temperature by 97 only out of 144 sera: the red cells of Case 2 were agglutinated by 142 out of 143 sera. The reactions of Case 2's red cells were stronger than those of Case 1 at this time. The agglutinin recovered in saline from the red cells of Case 1 six weeks previously still agglutinated at room temperature the fresh red cells of Case 1 and more strongly those of Case 2. The two sera absorbed with the red cells of Case 1 six weeks earlier gave no reaction with the fresh sample of Case 1: one serum did not react with the red cells of Case 2, the other gave only a very weak agglutination. It is probable, therefore, that the atypical agglutinogens in both cases were identical.

These two cases appear to be similar to those of Gaffney and Sachs in so far as their red cells could absorb from normal sera the agglutinins against themselves. They may differ from the case of Basil-Jones *et al.* in that the recovered agglutinin was specific at room temperature for the peculiar cells of the two patients and did not react with normal *O* cells. Gaffney and Sachs make no report on a recovered agglutinin.

Prof. Sachs kindly supplied us with a culture of Friedenreich's *M* bacillus which consistently, when grown in suspensions of red cells, leads to 'pan-agglutinability' of such suspensions by normal sera. Suspensions of three group *O* red cells were inoculated with *M.* bacilli; after nine days they were pan-agglutinable.

These cells were set up against six normal sera, saline extracts recovered from the agglutinated red cells of Cases 1 and 2, sera absorbed with the red cells of Case 1, and the sera of Cases 1 and 2.

The normal sera and the saline extracts of the patients' agglutinated cells both caused, at room temperature, strong agglutination of the transformed cells. The absorbed sera gave no agglutination with one of the transformed red cell suspensions and weak agglutination with the other two. The serum of Case 1 also showed a diminished agglutinating power, agglutinating one of the suspensions weakly but not the other two. The serum of Case 2, however, agglutinated almost as strongly as normal sera all three suspensions.

The results do suggest that the agglutinin in normal serum for the cells of these patients is related to the anti-*T* (of Friedenreich)[4]