

2. Fibre-length (inch)		
(a) By Bills Sorter	..	0.65
(b) By Bar Sorter	..	0.66
3. Fibre-length irregularity (%)		14.9
4. Fibre-weight per inch (Millionth of an oz.)	..	0.391
5. Fibre-strength (oz.)	..	0.221
6. Intrinsic strength	..	0.57
7. Ribbon width (thousandth of an inch)	..	0.98
8. Swollen diameter (thousandth of an inch)	..	1.49
9. Maturity (%)	..	77-14-9
10. Standard hair-weight (Millionth of an oz.)	..	0.380
11. Convolutions per inch	..	69
12. Wax Content (%)	..	0.212

It will be noticed that the cotton is a short stapled type, having a staple length of only about 5/8" but the most remarkable features about it are its high fibre-weight per inch, ribbon width and swollen diameter. Hitherto, nearly 5,000 samples of cotton have been tested at the laboratory, and in all probability the values of these three properties of this cotton are highest on record here; while owing to its high fibre-weight per inch, the intrinsic strength (the fibre-strength per unit fibre-weight per inch) is probably the lowest on record. Another remarkable feature about this cotton is its extremely low wax content, which again is the lowest on record in this Laboratory. This low wax content gives it a peculiar harsh feel, which is very characteristic of the cotton. Furthermore, for its length, this cotton possesses rather high fibre-length irregularity percentage and rather low percentage of mature fibres.

The cotton was also subjected to a spinning test in the Laboratory, and it was found that, even with a moderately high twist, the 6's warp yarns spun from it did not possess the requisite strength. This is due partly to its low staple length and partly to its high fibre-weight per inch. The cotton, however, though not so suitable for spinning purposes, would be excellent, owing to its harsh feel, for mixing with wool; also, provided it could be given a

softening treatment, it would form a suitable material for the manufacture of hospital lint after removing the small amount of wax associated with it.

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Biochemical Synthesis of Colouring Matter by an Indian Strain of *Penicillium* Mold

THE chemistry of colouring matters synthesised in higher plants has received considerable attention lately and the constitution of some have already been established. Colouring matters are also produced by some of the lower plants like molds and actinomycetes, the colour being usually located within the mycelium or cells and in a few cases it diffuses out into the culture medium. The colouring matters of the lower plants have not been studied systematically at all except in isolated cases by a few workers like Raistrick,¹ Friedheim,² Blochwitz,³ and Griegarieva-Manoilova and Pordieeva,⁴ etc. Amongst the *Penicillium* group of molds, although the fact that they have the capacity of producing pigments is known, systematic attempts at elucidating the constitution of the pigments or the mode of their synthesis are extremely rare, the only work in this field being that of Friedheim who has experimented with *Penicillium phœnicum*.³ This is probably due to lack of suitable micro-organisms which can produce pigments in experimentally workable amounts. Therefore the isolation from an indigenous source of a species of *Penicillium* which readily yielded scarlet coloured culture solutions has afforded an opportunity of studying the interesting problem of colour production. The present note is a preliminary report of the work so far done.

Whilst carrying out a survey of the microflora habitating on fruits of Northern India a mold was encountered on some of the samples of over-ripe William pears obtained from Kulu valley in the Punjab, which appeared to belong

to the *Penicillium* group of fungi and was an efficient producer of brilliant red to scarlet coloured pigment. It may be interesting to note that Galloway⁵ has found the acid soils of the hilly districts of India to be abundantly infested with *Penicillium* molds. In order to obtain the mold in pure culture the infected fruit was scraped and the scrapings shaken in physiological saline and the latter plated out in suitable decimal dilutions on oatmeal-maltose-agar or potato-maltose-agar medium. The discreet colonies of the chromogenic mold were picked out and replated after having been successively grown a few times on a medium consisting of: maltose 0.5%, asparagine 0.1% and potassium phosphate 0.01%. This medium is obviously unfit for the vigorous growth of yeasts and is therefore suitable for freeing the mold from yeasts and even fungi imperfectii which occur abundantly on the fruits.

Culturally the mold strongly resembled the *Penicillia*. On liquid media it has a greyish white occasionally yellowish growth. In forty eight hours the colour of the mycelium becomes green on account of the appearance of spores and the medium starts turning red. After 2-3 weeks the medium is deep red and the mycelium consists of dense closely woven hyphæ, the surface is deep green and the reverse velvety and red-brown in colour. On malt agar the vegetative growth is restricted, dense but irregular, spores abundant, conidial areas deep green, the medium rapidly turns deep red. The cultures on agar were sent to the centraalbureau voor Schimmelcultures Baarn Holland and have been stated to be identical with *Penicillium crateriforme* isolated by Gilman and Abbot⁶ in 1927 from the soils of Louisiana, Iowa and Utah States in America. It may be pointed out that climatically there is a resemblance between the above mentioned American States and the Indian district from where the present strain was isolated. The somewhat surprising fact should here be mentioned that Oxford and Raistrick⁷ in a study of biochemistry of '*Penicillium crateriforme* Gilm and Abb' in which they have used

the well-known Czapek-Dox medium have failed to notice any reddening of the culture solution even after twelve weeks; only the mycelium is stated by them to acquire in the later stages a faint pink or occasionally a brick-red colour.

The physiological needs of this mold are quite simple, a small amount of carbon source and a trace of nitrogen being all that is necessary. Such simple sources of carbon as ethyl alcohol, ethylene glycol, glycerine, isopropyl alcohol, acetone and lactic acid are assimilated although growth is more prolific with more complex carbohydrates. For the production of colour organic nitrogen compounds of intermediary complexity like amino-acids, peptides and peptones are more suitable nutrients than inorganic nitrogen on the one hand and albumins and proteins on the other. Amongst the sugars pentoses like arabinose and xylose are the most suitable substrates for colour production. Laevulose is converted slightly more readily than dextrose; galactose, mannose, sucrose and maltose and also to a lesser extent lactose readily yield the colouring matter, whilst trehalose, raffinose and to a small extent also dulcitol and mannitol give the red pigment. Dextrin gives little or no colour. In order to get comparable results in such experiments the coloured media were collected at the end of a definite number of days, filtered, made to known volume and the amount of pigment produced compared in the Lovibond tintometer. Triplicate lots of media were taken in each case in order to avoid experimental error which is otherwise very large. From the point of view of elucidation of mechanism of the pigment synthesis, it is naturally important to find out the simplest carbon compounds which can be utilised by the mold-enzymes in building up the complicated pattern of the pigment molecule and here experiments with pregrown mycelium have brought out the interesting fact that a number of simple carbon compounds can be converted into the colouring matter. The mold was first grown on a sucrose medium, the mycelium collected, washed well, cut into small

round pieces and placed on the surface of the new media under examination; precautions being taken during the whole operation to avoid extraneous infection. Blank tubes with distilled water were used as controls and their Lovibond tintometer values subtracted from those obtained with the actual media. In this way it has been found that the following substances readily give the pigment: methyl and ethyl alcohols, glycerine, amyl alcohol, ethylene glycol, tartaric, dihydroxytartaric, citric and mucic acids, and acetone. It is quite likely that synthesis of the pigment from the carbohydrates proceeds *via* any of these compounds.

For the large-scale production of the pigment sucrose has been selected as the substrate on grounds of convenience and economy. The medium contained in flasks consists of sucrose 0.5%, Bacto peptone 0.10% and potassium phosphate 0.01%, final pH after autoclaving or steam sterilisation at 100° C. was about 6. After inoculation with spores of the organism from an agar culture, the flasks were incubated at 30° C. Red colour begins to appear in about three days from the top and the colour slowly extends and the whole liquid turns red in one or two weeks, maximum reaching in about 2-3 weeks. The culture liquid is decanted off from the mycelium, filtered, mixed with 10-15% its volume of hydrochloric acid, left aside for half an hour and then extracted with about 30-35% its volume of amyl alcohol used in two portions. The amyl alcohol extracts are washed once with water, dried over sodium sulphate and the solvent distilled off *in vacuo*. The residual pigment is stirred up with a little ether or petroleum ether which dissolves out a brownish impurity, and the pigment is collected. This gives a deep red to chocolate coloured powder in a yield of about 0.100 gm. per litter of the culture solution. The crude pigment melts indefinitely between 180-200° C., can be adsorbed on suitable grades of alumina, and is precipitated from aqueous solutions with lead acetate. With ferric chloride no detectable deepening of colour occurs but strong acids or alkalis turn the bright red colour of an aqueous solution to an orange colour. It is oxidised to colour-

less form by boiling with hydrogen peroxide. It is insoluble in usual organic solvents, but dissolves readily in amyl alcohol from which it is not easily washed out with water. An aqueous solution appears deep red in ordinary light and greenish-brown under the mercury vapour lamp. The biochemistry of the synthesis of the pigment is being studied and its constitution will be examined when workable amounts have been prepared.

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¹ Raistrick, H., and co-workers, *Phil. Trans. (B)*, 1931, **220**, 209, 269, 297; *Biochem. J.*, 1932, **26**, 1907; 1933, **27**, 1170; 1934, **28**, 559, 1640; 1936, **30**, 1303; 1937, **31**, 385; *J. Chem. Soc.*, 1933, 488; 1937; 80.

² Friedheim, E. A. H., *Biochem. Z.*, 1933, **259**, 257; *Compte rend. Soc. Biol.*, 1933, **112**, 1030.

³ Blochwitz, A., *Ber. deutsch. bot. Ges.*, 1931, **49** (6), 319.

⁴ Grigorieva-Manoilova, O. C., Poradiceva, N. N. *Arch. Sci. Biol. (Petrograd)*, 1915, **19**, 119; *Abst. Bact.*, **7**, 333.

⁵ Galloway, L. D., *Ind. J. gr. Sci.*, 1936, **6**, 582.

⁶ Gilman, J. C., and Abbot, E. V., *Iowa State Coll. J. Science*, 1927, **1**, **3**, 225; *Biol. Abs.* 1928, **2**, No. 18643.

⁷ Oxford, A. E., and Raistrick, H., *Biochem. J.*, 1934, **26**, 1321.

Diet and Detoxication

DURING the present year, an investigation has been undertaken in this laboratory with a view to answer the question, "Can the rate of elimination of conjugated glucuronic acid be used to test the metabolic function of the liver?" In this connection, a large number of estimations were conducted, by an improved and comparatively recent technique,* on conjugated

* Salt's modification¹ of Tollen's naphthoresorcinol test has been used throughout these experiments. The final blue-violet colour of the ethereal extract was examined in a Pulfrich photometer, using filter, No. S. 53 with a wave length of 530 μ , and the result expressed in terms of the colour absorption (extinction co-efficient), which, according to Salt, (1935) is proportional to the quantity of glucuronic acid excreted.