stained in a similar manner to material prepared by the other silver techniques.

Overfixation is liable to bring about impregnation of the mitochondria and other inclusions as well as of the Golgi material. I have consistently used lithium carbonate to neutralize the formol; but I believe that any of the usual neutralizing agents would be equally suitable for the purpose.

I have successfully used this method to prepare mammalian tissues and the tissues of annelids, molluscs and insects. Referring to Aoyama's method, Gatenby and Beams[5] remark: "After having seen preparations of various vertebrate and invertebrate tissues made by this method, we have concluded that it is the best of the three formalin silver methods and the nearest approach to a good osmic Golgi technique". I find that preparations made with my cupric chloride formol method are not only as good as those prepared by Aoyama's method, but in many cases, chiefly in toned sections, the general appearance is superior. Cupric chloride has a slight advantage over cadmium chloride in being more easily available.

Like Aoyama's method, my technique is a modification of Cajal's formalin silver nitrate method. Cajal[1] originally suggested other nitrates such as those of manganese and lead, but these were found to be unsatisfactory. Da Fano[3] experimented with other salts, for example, cobalt sulphate and copper nitrate, but these did not give satisfactory results. I have tried different percentages of cupric chloride solution, but find that 1 per cent gives the best results. I have also treated tissues with saturated solution of cuprous chloride (its solubility in water is less than 0·5 per cent), but the preparations were inferior to those treated with cupric chloride.

I wish to thank Prof. R. A. R. Gresson for providing facilities for this work and for advice and encouragement.

D. S. Srivastava

Department of Zoology,
Queen's University, Belfast.
Oct. 29.

[1] Cajal, R. Y., *Trab. Lab. Invest. Biol. Madrid*, **12**, 127 (1914).
[2] Da Fano, C., *J. Roy. Mik. Soc.*, **40**, 157 (1920).
[3] Da Fano, C., *J. Physiol.*, **54**, 113 (1920).
[4] Aoyama, F., *Z. Wiss. Mikr.*, **46**, 489 (1929).
[5] Gatenby, J. B., and Beams, H. W., "The Microtomists' Vademecum" (Bolles-Lee) (11th edit., 1950), Chapter 32, see p. 413.

Detoxifying Mechanisms in Clothes Moth Larvæ

Larvæ of the clothes moth, *Tineola bisselliella* (Humm.), are able to develop on woollen fabric impregnated with many of the inorganic poisons which are highly toxic to insects and other animals.

Salts of those metals capable of producing insoluble sulphides result in the formation of characteristically coloured sulphides in the food undergoing digestion in the midgut. The alkaline (*p*H 10), highly-reducing (about −300 mV.) midgut digestive juices[1,2] reduce the disulphide bonds of the cystine of wool (present to the extent of about 13 per cent), and sulphydryl groups and hydrogen sulphide are formed. Sulphides are formed by reaction of metal ions with these groups. Larvæ fed on metal-impregnated silk (which contains no sulphur) do not produce sulphides unless a sulphur-containing material (such as cystine, methionine, or glutathione) is added to the diet. This detoxifying mechanism is reminiscent of the protection given by 2.3-dimercapto propanol (BAL) against poisoning by arsenic and a number of other metals[3].

When a sulphide-forming metal is present in the diet, less cystine is excreted than on a metal-free diet. Most of the metal sulphide formed is excreted. However, a small amount forms a colloidal solution with the amino-acids or polypeptides liberated by digestion of the food or present in the digestive secretions. Sulphides are then taken up by the midgut epithelium and accumulated in the cavities of the goblet cells (hitherto of unknown function) in the anterior and posterior regions until the following moult, when the entire midgut epithelium is cast off and regenerated.

A number of elements are incapable of forming insoluble sulphides under the conditions existing in the digestive tract. Of these, the alkaline earths are deposited, initially as insoluble phosphates, in the columnar cells of the anterior and posterior midgut. It is probable, also, that small quantities of absorbed fluoride are deposited with calcium in these granules.

Fuller details of the poisons used and the detoxifying mechanisms involved will be published elsewhere[2].

D. F. Waterhouse

Division of Entomology,
Commonwealth Scientific and
Industrial Research Organization,
Canberra. Oct. 30.

[1] Linderstrøm-Lang, K., and Duspiva, F., *C.R. Lab. Carlsberg*, **21**, 53 (1936).
[2] Waterhouse, D. F., [*Aust. J. Sci. Res.*, B, **5** (1) (1952)].
[3] Danielli, J. F., Danielli, M., and Mitchell, P. D., *Biochem. J.*, **41**, 325 (1947). Stoker, L. A., *Biochem. J.*, **41**, 358 (1947). Thompson, R. H. S., and Whittaker, V. P., *Biochem. J.*, **41**, 342 (1947).

Natural Occurrence of Rough Variant of a Yeast, *Candida albicans*

In the course of an investigation by Dr. M. J. Marples, of this Department, and myself upon the effects of antibiotics upon the yeast flora of the mouth, *R* colonies of *Candida albicans* have been found upon repeated occasions on primary cultures which had been incubated at 37° C. for two days. The occurrence of dissociated, rough variants of *Candida albicans*, which show a reduced pathogenicity to rabbits, has been noted in the literature since 1935. To our knowledge all these variants have been reported as occurring in old laboratory cultures maintained upon artificial media for periods of years, or else have been induced more rapidly by special conditions such as treatment with immune serum and lithium chloride. Our cultures were made by plating saline mouth-washings upon Sabouraud agar of *p*H 4. Continuous variation was observed, from colonies differing only by a central depression from normal smooth colonies upon the same plate, to smooth colonies haloed by a ring of mycelium in the medium, and to colonies so raised, rough and warty that they appeared to be, on casual inspection, of a wholly different species. These isolates upon appropriate media showed the typical chlamydospore formation and biochemical characters of *C. albicans*.

That these *R* forms were occurring naturally in the oral cavity, and were not induced by the unusually acid medium used to suppress bacterial growth, is suggested by the fact that, of the twelve patients carrying *C. albicans* who were under observation, only four produced *R* variants, and that these appeared in small numbers upon repeated cultures. Further, Langeron and Guerra[1] regard an excessively