Kirimura's method with the view of getting an active principle from the small number of brains.

The complexes of brains and subœsophageal ganglia from pupæ of *Bombyx* that had been mostly shortly before the completion of adult development were stored in a deep-freezer at − 18° C. and used as material to be extracted. Brains were counted before crushing to facilitate an easy computation of dose per test pupa. All the work was carried out at room temperature.

240 brains (fresh wt. 97 mgm.) were homogenized with 1 ml. of methanol and centrifuged. The same extraction was repeated twice more with the residue. The extracts thus obtained were combined and dried *in vacuo*. A small amount of yellow matter thus prepared was dissolved in 2 ml. of distilled water plus 5 ml. of ether, the latter being removed after thorough shaking. The water layer was washed with ether twice more and a trace of ether remaining in the water was removed *in vacuo*. The water fraction thus obtained was made up with distilled water to 2 ml., 0·1 ml. of which was injected into each of 20 test pupæ of *Philosamia*. 19 out of the 20 pupæ metamorphosed into moths 18–24 (average 19) days later at 25° C. (At this temperature normal pupæ can emerge as moths 17–19 days after pupation.) The combined ether washings were dried also *in vacuo* to yield yellow oily matter. It was dissolved in 2 ml. of sesame oil and 0·1 ml. of the solution was injected into each of 20 test pupæ with the result that not one of them indicated an appreciable differentiation toward adult. Control experiment of injecting water or sesame oil alone proved also to be ineffective. A repetition of the same experiment yielded essentially the same result.

The active principle was also prepared by extraction with 2 per cent sodium chloride solution. 350 brains were homogenized with 0·7 ml. of the saline solution and centrifuged at 9,000 r.p.m. for 30 min. The same procedure was repeated three times more with the residue, and the extracts thus obtained were about 2·8 ml. All 37 pupæ, in which 18 pupæ were injected each with 0·1 ml. of the extract and 19 with 0·05 ml., were able to metamorphose into moths 18–25 (average 20) days and 19–25 (average 21) days later respectively. Injection of 0·1 ml. and 0·05 ml. of the saline solution brought about no sign of adult differentiation.

Thus it is evident that the active extract of the brain hormone can be prepared successfully from a relatively small number of brains. Here it must be emphasized that there is an important difference between our finding and that obtained by Kobayashi and Kirimura: namely, our principle is water-soluble, while that obtained by Kobayashi and Kirimura is ether-soluble, as tested by the water–ether partition. Further purification now in progress will give further information about the chemical nature of the brain hormone.

We are indebted to Dr. S. Morohoshi, director of Gunze Silkworm and Mulberry Research Institute, and Dr. S. Sasaki, director of Ayabe Branch of Sericultural Experimental Station, who supplied the silkworm pupæ.

Mamori Ichikawa
Hironori Ishizaki

Zoological Institute, College of Science,
University of Kyoto.

[1] Williams, C. M., *Biol. Bull.*, **110**, 201 (1956).
[2] Karlson, P., in *Proc. Fourth Intern. Cong. Biochem.*, edit. by Levelbook, L., 37 (Pergamon Press, 1959).
[3] Kobayashi, M., and Kirimura, J., *Nature*, **181**, 1217 (1958).

GENETICS

Sterile Crosses between Mutations of *Tribolium confusum* Duv.

'McGill Red' is a wild strain of *T. confusum* maintained by me for some thirty years. 'McGill Black' is a jet black mutation which arose in the wild strain in 1950. The life-histories of both strains have been described by H. M. Slatis and me[1]. In the spring of 1960 another black mutation which may be called for convenience 'Slough Black' was received from the Pest Infestation Laboratory at Slough. An extensive programme of research has been under way to study the genetics of 'Slough Black' in relation to 'McGill Black' and 'McGill Red'.

It has been found that, when females of 'McGill Black' are mated to any male other than that of 'McGill Black', numerous eggs are produced, but these are all non-viable, no development occurring. The males of all the genotypes obtainable from crosses between the three strains have been mated with females of 'McGill Black' with similar results.

The eggs have been sectioned, but there is no trace of development, and eventually the eggs dry up and wither. Superficially they appear quite normal. There would seem to be four possible causes: (*a*) the matings are ineffective, no sperms being transferred; (*b*) there is some genetic lethal effect; (*c*) there is a cytoplasmic defect; (*d*) there is the faint possibility that rearing the insects on flour plus yeast has had an effect. Further work is being done on this problem, and some stocks are being reared on flour plus wheat-germ to check (*d*).

John Stanley

Department of Zoology,
McGill University,
Montreal, Quebec.

[1] Stanley, J., and Slatis, H. M., *Ecology*, **36**, 473 (1955).

5-Methyltryptophan and Darkening of the Hair in Yellow A^y/a Mice

Previous experiments have shown that powdered preparations of the skin and hair of yellow (A^y/a) mice oxidize L- (but not D-) tryptophan and also DL-5-hydroxytryptophan with the production of yellow solutions[1,2]. To obtain further evidence on the question of whether tryptophan, or a derivative, might be implicated in the production of yellow pigment in the hairs of this strain of mice, *in vivo* experiments were carried out using an antimetabolite of tryptophan[3]. Starting at 3–4 days of age, daily subcutaneous injections of DL-5-methyltryptophan were made in these mice for a period of 7–10 days. This is a period of vigorous pigment production. (Mice were obtained from the R. B. Jackson Memorial Laboratories, Bar Harbor, Maine. DL-5-methyltryptophan was a product of Sigma Chemical Co., St. Louis, Mo.)

While lower doses gave equivocal results, the injection of 1 mgm./gm. DL-5-methyltryptophan into these suckling mice daily for a week (a dosage high enough to cause measurable depression in growth-rate) resulted in animals the coats of which were markedly less yellow than those of animals given saline or L-tryptophan daily (Table 1). Instead, coats of experimental animals appeared darker in colour than those of controls. When unstained 10μ cross-