

embryos disappears gradually with advancing development<sup>9</sup>, and it has been found in this laboratory that the non-incubated chick embryo has ribosome-associated basic proteins.

Finally, as can be seen in Fig. 2, the adult histone pattern is different in different organs. In erythrocytes and liver the relative amount of  $F_3$  is intermediate, while in the other organs  $F_3$  is very near in quantity to  $F_2$ . In electropherograms the histone pattern is different in erythrocytes, spleen and testes from chicken<sup>10</sup>.

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- <sup>1</sup> Stedman, E., and Stedman, E., *Nature*, **166**, 780 (1950).  
<sup>2</sup> Leisle, I., *Nature*, **189**, 260 (1961).  
<sup>3</sup> Bloch, D. P., *J. Histochem. Cytochem.*, **10**, 137 (1962).  
<sup>4</sup> Allfrey, V. G., Littau, V. C., and Mirsky, A. E., *Proc. U.S. Nat. Acad. Sci.*, **49**, 414 (1963).  
<sup>5</sup> Moore, B. C., *Proc. U.S. Nat. Acad. Sci.*, **50**, 1018 (1963).  
<sup>6</sup> Lindsay, D. T., *Science*, **144**, 420 (1964).  
<sup>7</sup> Agrell, I. P. S., and Christensson, E. G., *Nature*, **191**, 284 (1961).  
<sup>8</sup> Johns, E. W., Phillips, D. M. P., Simson, P., and Butler, J. A. V., *Biochem. J.*, **77**, 631 (1960).  
<sup>9</sup> Agrell, I. P. S., *Pathol. Biol.*, **9**, 775 (1961).  
<sup>10</sup> Neelin, J. M., and Butler, G. C., *J. Biochem. Physiol.*, **39**, 488 (1961).

### Benzyl Thiocyanate Taint in the Milk of Dairy Cattle Ingesting *Coronopus didymus* Sm.

AN objectionable flavour is found in the milk of dairy cattle which graze on pastures infested with the cruciferous weed *Coronopus didymus* Sm. (bitter cress or land cress)<sup>1</sup>. This taint is of considerable concern in Queensland and New Zealand where the weed is widespread during winter; unlike many other fodder or weed taints in milk, it cannot be removed during the manufacture of cream or butter<sup>1,2</sup>. The taint in milk is characteristically a burning flavour with a sharp odour, while in butter it is a burnt flavour with a burning after-taste.

In earlier investigations<sup>2,3</sup> glucotropaeolin, a mustard oil glucoside<sup>4</sup>, was found to occur in this plant. Benzyl cyanide, benzyl isothiocyanate, dibenzyl disulphide and benzyl mercaptan have been isolated as products of the enzymatic or chemical breakdown of the glucoside. None of these compounds was found to give rise to the characteristic taint when fed to dairy cattle<sup>1,3</sup>, or when added to taint-free milk.

Recently, Virtanen *et al.*<sup>5,6</sup> found that on crushing the seeds of the cruciferous weed *Lepidium sativum* L. and incubating the crushed seeds in water, benzyl isothiocyanate is the initial breakdown product of the glucotropaeolin which is present in the plant. The isothiocyanate, however, was found to isomerize rapidly in the incubated extract, to give benzyl thiocyanate. This isomerization, observed also in a related *Lepidium* species, was apparently carried out by an enzyme present in the crushed seeds.

Similar experiments have now been carried out on seeds from *C. didymus*. Gas chromatograms and infra-red absorption spectra of extracts of the crushed seeds, after chromatography on alumina, have indicated that benzylthiocyanate ( $\nu$ -SCN 2158  $\text{cm}^{-1}$ ) (ref. 7) is produced in *C. didymus* by isomerization of initially produced benzyl isothiocyanate. The isomerization was apparently complete within 15 min of mixing the powdered seeds with water.

The characteristic off-flavour has now been reproduced in the milk of dairy cattle by either drenching a dairy cow with benzyl thiocyanate dissolved in peanut oil or by the addition of benzyl thiocyanate to taint-free milk. When a dairy cow was fed 3 g benzyl thiocyanate, a taint developed in her milk within 2 h, became very strong after 4–6 h and disappeared within 24 h. The characteristic taint carried through to the derived cream and butter. Addition of

0.16 p.p.m. of benzyl thiocyanate to taint-free milk produced a detectable taint, whereas 4 p.p.m. gave a very strong taint to the milk, and the characteristic burning flavour to the cream.

There is the possibility that benzyl mercaptan may contribute to the associated taint in butter, as has previously been suggested<sup>3</sup>, since benzyl thiocyanate could be reduced to the mercaptan by cysteine or similar reducing agent, during the pasteurization of cream before the manufacture of butter.

Evaluation of flavour of the milk and products in these experiments was carried out by experienced dairy produce graders from the relevant Queensland and Commonwealth Government Departments.

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- <sup>1</sup> Conochie, J., *Austral. J. Dairy Technol.*, **5**, 43 (1950).  
<sup>2</sup> McDowall, F. H., Morton, I. D., and McDowell, A. K. R., *N.Z. J. Sci. Technol.*, **A**, **28**, 305 (1947).  
<sup>3</sup> Forss, D. A., *Austral. J. App. Sci.*, **2**, 396 (1951).  
<sup>4</sup> Ettliger, M. G., and Lundeen, A. J., *J. Amer. Chem. Soc.*, **79**, 1764 (1957).  
<sup>5</sup> Gmelin, R., and Virtanen, A. I., *Acta Chem. Scand.*, **13**, 1474 (1959).  
<sup>6</sup> Virtanen, A. I., and Saarivirta, M., *Suomen Kemistilehti*, **B**, **35**, 102 (1962).  
<sup>7</sup> Mazzucatto, A., Foffani, A., Illiceto, A., and Svegliardo, G., *Adv. Molec. Spectr.*, **2**, 861 (1962).

### Mechanism of the Inhibitory Effect of Adenine Nucleotides on Porphyrin Synthesis by *Rhodospseudomonas spheroides*

EXCESS biosynthesis of porphyrins is strongly decreased by some adenine nucleosides and nucleotides (inosine, 5' AMP, 5' ADP, 5' ATP) *in vivo* as well as *in vitro*<sup>1-3</sup>. Experiments with *Rhodospseudomonas spheroides* have shown that ATP is the most active of these adenine derivatives, determining a more than 90 per cent inhibition<sup>2,3</sup> in a concentration of 3 m.moles/l. of medium. On the other hand, it was established that this effect is confined to the step of ALA formation<sup>2,3</sup>.

To explain this decrease of porphyrin synthesis we have first envisaged, on the basis of the tricarboxylic-glycine cycle of Shemin<sup>4</sup>, a negative feed-back reaction on ALA synthetase. Our recent experiments have shown that this hypothesis is not correct. In *Rhodospseudomonas spheroides* grown anaerobically for 24 h in the light in medium I of Lascelles<sup>5</sup>, ALA synthetase activity was even enhanced when ATP was added to the medium (Table 1).

Table 1. ALA SYNTHETASE ACTIVITY OF EXTRACTS IN  $\mu$ MOLES ALA FORMED PER MG PROTEIN OF EXTRACT\*

Exp.	Extracts of micro-organisms grown in presence of ATP (3 m.moles/l. of medium)	Extracts of micro-organisms grown without addition of ATP to the medium
1	66.6	18.3
2	45.0	11.4
3	23.0	4.8

\* 0.5 ml. extract was incubated for 30 min at 37° C and pH 7.4, with glycine, succinate, CoA, pyridoxal, Mg<sup>++</sup>, and ATP (following the technique of Granick and Urata<sup>6</sup>).

We have therefore assumed that the adenylic derivatives enhance the formation of a physiological inhibitor of porphyrin synthesis, the existence of which in *Rhodospseudomonas spheroides* was reported by Kikuchi and colleagues<sup>7</sup>. This hypothesis seems to be confirmed by our experiments. *Rhodospseudomonas spheroides* suspended in Lascelles medium I to which ATP was added (3 m.moles/l. of medium) were incubated for 24 h semi-anaerobically, in the light. The micro-organisms were then gathered by centrifugation at 0° C and repeatedly washed with 0.9 per cent NaCl solution.