

reduction (cinnamic acid  $\rightarrow$  hydrocinnamic acid). The presence of acetophenone, which is rather rare in plant material, may be explained by a mechanism found by Dakin<sup>8</sup> which implies  $\beta$ -oxidation and decarboxylation of hydrocinnamic acid.

The *p*-ethyl-phenol, the principal phenol of the beaver gland, may be derived from tyrosine by deamination and decarboxylation. The great similarity of the constituents of the castoreum with substances frequently found in the urine of vertebrates made us think that the urine of vertebrates might also contain *p*-ethyl-phenol. We distilled the phenols of an industrial extract of hydrolysed pregnant mare's urine and actually isolated 32 gm. of *p*-cresol (see Marshall<sup>9</sup>) and 4.2 gm. of *p*-ethyl-phenol (identified as phenylurethane m.p. 117° and as *p*-ethyl-phenoxyacetic acid, m.p. 95°)<sup>10</sup>.

Baumann<sup>11</sup> had already suggested in 1879 the following scheme of degradation of tyrosine by intestinal bacteria: tyrosine  $\rightarrow$  *p*-oxyphenyl-propionic acid  $\rightarrow$  *p*-ethyl-phenol  $\rightarrow$  *p*-oxyphenyl-acetic acid  $\rightarrow$  *p*-cresol *p*-oxybenzoic acid  $\rightarrow$  phenol. All these substances have already been isolated from urine, except *p*-ethyl-phenol.

The *p*-propyl-phenol found in the castoreum may have a similar origin, implying reduction of the carboxyl group of tyrosine; but we have not yet succeeded in isolating it from the phenols of pregnant mare's urine.

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<sup>1</sup> Stevens, P. G., *J. Amer. Chem. Soc.*, **65**, 2471 (1943).

<sup>2</sup> *Bull. Soc. Chim. biol. (Trav.)*, **23**, 1457 (1941).

<sup>3</sup> *Bull. Soc. Chim. biol. (Trav.)*, **24**, 1155 (1942).

<sup>4</sup> Lederer, E., and Polonsky, J., *Bull. Soc. Chim. biol. (Trav.)*, **24**, 1386 (1942).

<sup>5</sup> *Bull. Soc. Chim. biol. (Trav.)*, **25**, 1073 (1943).

<sup>6</sup> *Bull. Soc. Chim. biol. (Trav.)*, **25**, 1381 (1943).

<sup>7</sup> Osa, A., *Ann. Chim.*, **11**, 1 (1940).

<sup>8</sup> Sin, F. D., *J. Biol. Chem.*, **6**, 217 (1909); **9**, 123 (1909).

<sup>9</sup> Marshall, P. G., *Nature*, **140**, 362 (1937).

<sup>10</sup> *Bull. Soc. Chim. biol. (Trav.)*, **25**, 1237 (1943).

<sup>11</sup> Baumann, E., *Ber.*, **12**, 1452 (1879).

#### A Substitute for 'Annatto' in Butter

BUTTER—especially that prepared from buffalo milk—is usually artificially coloured to make it look like that afforded by well-known dairy breeds of cows (such as the Jerseys). Many colouring matters for butter are sold under different names, the commonest of which is that known as 'annatto', which is an oil extract of seed coats of *Bixa orellana*. This, as well as many other less common colouring matters, have always been imported into Egypt, but during the War butter makers were forced to prepare butter without artificial colouring matter.

Several trials have been made to cultivate the plant *Bixa orellana* in Egypt, but a good yield will not be expected for many years. It therefore seemed of interest to attempt to prepare, from plant materials available in Egypt, a new colouring extract which may be used as a substitute for 'annatto' and similar colouring matters. A sample of a fresh oil extract of 'annatto' was examined by the tintometer and the proportions of yellow and red colour units determined. Many coloured plant parts containing adequate yellow and red pigments were studied with the aim of finding which of them would provide, either singly or in combination, the proper shade for a new colouring matter. Many organic solvents were also used for the extraction of the colouring matters from the various powdered plant tissues. The results of the extractions and analysis by the Lovibond tintometer showed that none of the plant parts examined could be used singly for the preparation of the proposed colouring extract. It was further shown that one and the same plant part showed different proportions of yellow and red units according to the solvent used as extractant, and also according to the method of drying the plant material before extraction. The best extracts for the preparation of the suggested colouring matter are red peppers (*Capsicum annuum* var. *grossum*) in ether, and the rhizome of *Curcuma tinctoria* in ethyl alcohol. The analysis of the latter extracts by the tintometer enabled us to calculate the exact amounts of both the *Capsicum* fruits and the *Curcuma* rhizome which, when extracted with the appropriate solvents and mixed together, would give the same proportion of yellow and red colour units as that found in 'annatto'.

For the preparation of a sample of this annatto-like colouring matter, 7.5 gm. of dry fruits of red peppers (dried first in the sun and then in an oven at 60° C.) are extracted with ether and the extracted colouring matter is then taken up into 50 c.c. of cotton seed oil. Simultaneously, 6.9 gm. of *Curcuma* rhizome (oven-dried and powdered) are extracted with absolute alcohol and the colouring matter is taken up into 50 c.c. of cotton seed oil. On mixing these two oil extracts thoroughly we get 100 c.c. of a coloured oil which contains yellow and red colour units equivalent to those found in 100 c.c. of oil extract of annatto.

We studied the stability of the suggested extract in lights of different wave-lengths and at different temperatures and found it most stable in red light or in complete darkness. Refrigeration was of too little value to warrant its use for storage of the extract. It may be noted that in ordinary diffused light the yellow and red colour units disappeared more rapidly from the annatto extract than from the substitute colouring oil.

The new colouring extract has been tested and used by the Dairy Department, Faculty of Agriculture, Fouad I University, as well as many dairy farms in Egypt, and has given satisfactory results.

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#### A Photosensitized Keratitis in Cattle Dosed with Phenothiazine

SINCE the discovery of the anthelmintic properties of phenothiazine, it has been used widely in New Zealand for the treatment of young sheep and cattle.

During the past year there has been brought to our notice, by field veterinarians and inspectors of stock, the occurrence of a number of cases of corneal opacity and temporary blindness in calves a few days after ordinary therapeutic doses of phenothiazine. We have definite records of its occurrence on twenty-five farms; only one or two animals may react, but in one instance every calf in a mob of seven was affected. The symptoms observed the day after dosing were profuse lachrymation and sometimes a slight oedematous swelling of the skin around the eye and including the upper eyelid. Between 36 and 60 hours after dosing there appeared, in one or both eyes, a diffuse opacity the position of which was characteristically between the centre of the cornea and its lateral border. There was little loss of appetite, and photophobia, though present, was not very marked. In some animals the lesions disappeared within ten days, but in more severely affected cases a marked oedema of the epithelium and sub-stantia propria developed and ulceration occurred about the fourth or fifth day. Some of these animals still exhibited a slowly resolving lesion after three or four months. Similar ocular lesions have been referred to in accounts of phenothiazine poisoning in pigs<sup>1,2</sup>, but there is no record of its occurrence in cattle or other animals.

Experiments to study the condition are in progress at Wallaceville, using young calves as experimental animals. Lesions have been produced in calves dosed with several samples of the drug, with and without added wetting and suspending agents, and of British, American and Australian manufacture. A recrystallized sample gave similar results.

Evidence obtained from field cases and from controlled experiments shows clearly that bright sunlight is necessary for the development of the lesions, although marked signs of photosensitization of the skin are not present. Lesions fail to develop in dull weather or in stalled animals. When one eye is covered by a black velvet shield, lesions do not occur in that eye. Furthermore, the characteristic position of the lesion is on that portion of the cornea which is most exposed to light during grazing. When animals are restrained in a lateral recumbent position, the lesion develops above the centre of the cornea, again the area receiving most sunlight.

Photosensitization by phenothiazine derivatives has been studied in man<sup>3</sup> and in pigs<sup>4</sup>, but there is no record of its occurrence in sheep, and negative experimental results were obtained by Swales<sup>5</sup>.

Corneal lesions are not a recognized feature of photosensitivity diseases. However, we have produced typical corneal lesions in calves by injecting hæmatoporphyrin into the anterior chamber and afterwards exposing the eyes to sunlight, and similar results were obtained by the injection of phenothiazine derivatives (thionol, phenothiazone, and the leuco phenothiazone conjugate isolated from sheep urine). Derivatives of phenothiazine, not yet definitely identified, have been detected in the aqueous humour and tears of dosed animals, but concentrations were considerably lower than contemporary blood levels.

Corneal opacities and also photosensitization of the shaved skin have been produced in a light-coloured calf dosed with phenothiazine and afterwards exposed to radiation from an impregnated-carbon arc-lamp passing through windowglass and a Wood's-glass filter. From these results it appears likely that the effective radiation lies between 320 and 380 m $\mu$ .

A fuller account of the observations recorded above is to be published elsewhere, and further attempts are being made to identify the particular substance responsible.

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<sup>1</sup> Thorning, W. M., Morrill, C. C., and Boley, L. E., *Vet. Med.*, **37**, 120 (1942).

<sup>2</sup> Britton, J. W., *Cornell Vet.*, **33**, 368 (1943).

<sup>3</sup> De Ede, F., Wilson, R. H., and Thomas, J. O., *J. Amer. Med. Assoc.*, **114**, 2095 (1940).

<sup>4</sup> Swales, W. E., Albright, W. D., Frazer, L., and Muir, G. W., *Can. J. Comp. Med.*, **6**, 169 (1942).

<sup>5</sup> Swales, W. E., *Can. J. Comp. Med.*, **4**, 164 (1940).

#### Anopheline Life-Cycles and Population Fluctuations

INVESTIGATION of a population of adult *Anopheles melas* (= *A. gambiæ* var. *melas*), conducted during 1941, showed that their numbers underwent regular fluctuations, with very marked peaks at eight-day intervals, and that the only exceptions to this rule were when the rhythm was broken by the dominating influence of tides<sup>1</sup>. At the time it was difficult to suggest a reason for the fluctuations, because work on both *A. melas* and its close relative *A. gambiæ*, by various people, indicated that the life-cycle must occupy at least ten days. Similar fluctuations were found in an *A. funestus* population at twelve- to fourteen-day intervals, although the supposed time for its complete life-cycle was about twenty days. I tentatively suggested that the fluctuations might be due to intraspecific competition, although this explanation seemed most unlikely.

No. 2 Entomological Field Unit, R.A.M.C., has now conducted investigations in Assam which show that a population of *A. minimus* undergoes regular fluctuations at five- and ten-day intervals, and these fluctuations are only upset by occasional very heavy storms (1–2 in. a day), which delay the peak by one or two days if they occur nine days before the peak is due. Larval investigations, conducted simultaneously, have shown that the life-cycle in Nature at this time occupies ten days, with larval and pupal peak populations which fit and accord with the adult population peaks.