LETTERS TO THE EDITORS

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Chemical Taxonomy

THE differences between species of animals and of plants are ultimately chemical, and Ford[1] has shown in butterflies that classification based upon morphology corresponds with chemical differences in pigments. The distinction between two species within a genus must be due, in part at least, to differences between their proteins. Such differences cannot be shown by ordinary chemical methods, but in the case of one protein, namely, hæmoglobin, differences have lately been found by spectroscopic means between several species of the crustacean genus *Daphnia*[2]. In this genus it has long been a matter of discussion as to whether the species *D. obtusa* Kurz is really worthy of specific rank or is merely a variety of *D. pulex* (De Geer). Scourfield has, however, recently discovered a new, clear-cut morphological distinction between the two forms which confirms the specific rank of *D. obtusa*[3]. In these circumstances it is particularly interesting to find that their hæmoglobins also differ[2]. Another such instance of spectroscopic evidence in a case of doubtful specificity can now be reported.

Besides the common British species of the marine polychæte genus *Sabella*, namely, *S. pavonina* Savigny, there is found on our coasts an uncommon form known as *S. pavonina* var. *bicoronata* Hornell. Unlike the typical *S. pavonina*, this variety has a crown with its two sides unequal in size, although less unequal than in the case of *Spirographis spallanzanii* Viviani. The variety *bicoronata* thus appears at first to be intermediate between *Sabella pavonina* and *Spirographis spallanzanii*. The status of the three forms has now been re-examined by Ewer[4], who concludes that *Spirographis* is so close morphologically to *Sabella* that it does not merit generic rank. He therefore abolishes the former genus. Ewer confirms the status of *bicoronata* as a variety of *Sabella pavonina*, not promoting it to a species intermediate between *S. pavonina* and *S. spallanzanii*; for this decision he makes use both of external and blood vascular characters.

Twenty years ago, I compared the chlorocruorin absorption spectra of *Sabella pavonina* and *S. spallanzanii*, using the Hartridge reversion spectroscope, and found them to differ both in the wave-length of the axis of the α-band of their oxychlorocruorins and in their span (that is, the difference between the α-band in the oxy- and carboxy-compounds)[5]. It now becomes of interest to obtain the corresponding values for the variety *bicoronata*, in order to see whether they coincide with the values for *S. pavonina* or are intermediate between those for the two *Sabella* species. I have therefore repeated my measurements for *S. pavonina* and have obtained new data for var. *bicoronata*. The same spectroscopic precautions were taken as those lately described[6], and in addition another most important routine procedure, which should then have been mentioned, was always followed. For unknown subjective reasons, there are days when the wave-length values obtained for known absorption bands are higher, or on other days lower, than usual, by one or several Angström units. This unavoidable error can be eliminated by measuring the α-band of the observer's own oxyhæmoglobin, using the standard dilution of clear laked blood, with standard illumination and slit opening. This measurement is made at the beginning and again at the end of each day's investigation, and the excess or deficit from the average value for oxyhæmoglobin (this average being known from numerous previous measurements) is added to or subtracted from the values found that day for the unknown substances.

I find that in var. *bicoronata* the α-band of oxychlorocruorin has a wave-length 8 A. shorter than in *S. pavonina*, the value of which is 6061 A. Previously, I had found the value for *S. spallanzanii* to be 11 A. shorter than for *S. pavonina*, so that in this respect var. *bicoronata* is nearer *S. spallanzanii* than *S. pavonina*. On the other hand, the spans of var. *bicoronata* and *S. pavonina* turn out to be the same as one another, whereas the spans of *S. spallanzanii* and *S. pavonina* had been found[5] to differ by 16 A. The spectroscopic data show, then, that as regards one protein at least, var. *bicoronata* differs from the typical *S. pavonina* and approaches *S. spallanzanii*.

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Feb. 5.

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An Antibiotic from *Spiræa aruncus* L.

FOLLOWING the observation[1] that a watery extract of leaves and flowers of *Spiræa aruncus* L. possesses antibacterial properties, the active substance has now been isolated from the plant in crystalline form. It has, however, very low activity, preventing the growth of *Staph. aureus*, *B. proteus* and *B. coli* in a dilution of only 1 in 4,000, and having no effect on *Ps. pyocyanea* at 1 in 2,000. In view of its low activity, the substance has not been investigated in detail, but it is perhaps worth putting on record the following results.

An aqueous extract was prepared by grinding the fresh leaves and flowers with sand and distilled water and pressing out the fluid. The latter was adjusted to *p*H 3, boiled and centrifuged. The supernatant liquid was extracted three times with equal volumes of ether, which were pooled, concentrated by distillation and passed through a column of acid-washed alumina (final *p*H about 5·0). The antibiotic was situated in a band in the middle of the column, from which it was eluted with phosphate buffer *p*H 6·5, and afterwards extracted into ether. On evaporation of this ethereal extract there remained an orange-coloured oil. Extraction of the oil with hot benzene gave a solution from which the active substance separated in crystalline form on cooling. It was recrystallized by the addition of benzene to its solution in chloroform, and formed fine colourless prisms, m.p. 79–80° C. $[\alpha]_D^{20}$ (in water) + 55·8°.

The results of elementary analysis and molecular weight determination (Barger method) indicated that the molecular formula of this substance was $C_{10}H_{14}O_4$. The molecule contained 0·8 C-methyl groups according to the Kuhn–Roth determination. O-methyl was absent.

In the presence of palladium charcoal catalyst, the substance absorbed six atoms of hydrogen. In solution in a mixture of carbon tetrachloride and chloroform there was a rapid addition of two atoms of bromine.

A solution of the substance in pyridine gave a weak reddish-brown colour with sodium nitroprusside[2]. It did not reduce Tollen's reagent at room temperature, but produced an immediate heavy precipitate of silver from a solution of ammoniacal silver nitrate containing caustic soda[3].

The active substance was neutral and showed no ketonic properties. On treatment with cold alkali, however, by which its antibacterial activity was destroyed, one acidic group appeared; at the same time, ketonic properties became detectable, for Brady's reagent then precipitated an acidic 2·4 dinitrophenylhydrazone from the acidified solution.

Some of the properties of this antibiotic suggest that an α-β unsaturated lactone ring may be present in its molecule.

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A Light-Sensitive Enzyme in Cow's Milk

WHILE during the natural traffic between dam and suckling there is no exposure of the milk to daylight, under dairy industrial conditions there is often very considerable exposure. The effect of daylight or sunlight on milk has not received much experimental attention, though Kon and Watson[1] showed in 1936 that light brought about the oxidation of vitamin C in milk.

In experiments published in 1938[2], it was shown that an enzyme which hydrolyses tributyrin was present in all samples of fresh cow's milk examined. The determination of the lipase activity of milk samples was usually carried out immediately they were received from the cow-house, particularly since it was observed that, if they were kept (in glass bottles) on the laboratory bench for a few hours, variable losses of up to 20 per cent of the original lipase activity might be incurred. Whereas these losses showed no clear correlation with temperature, they could usually be avoided by storing the milk in the ice chest. In experiments done conjointly with the late Dr. E. C. V. Mattick just before the War, it appeared highly likely that this effect was due to the sensitivity of the lipase to visible light.

This investigation had to be put on one side owing to war urgencies, but it has been taken up again recently, and there is no doubt that the lipase (tributyrinase) of cow's milk is quite sensitive to sunlight. Exposure of fresh milk in glass vessels (depth of milk approximately 1 cm.) to bright summer sunshine for 10 minutes destroys 40 per cent of the enzyme (milk at a temperature under 5° C.), while exposure for 30 min. achieves about 80 per cent destruction. Thirty minutes exposure on the laboratory window-sill, but out of direct sunlight, may cause 30 per cent destruction of the lipase.

A source of ultra-violet light will also readily bring about the destruction of the enzyme in milk. Half an hour exposure to an 800-watt (220-volt) quartz mercury vapour lamp at a distance of 15 cm. destroys three quarters of the lipolytic power of the milk. The interposition of a piece of half-inch plate-glass affords little protection; it would appear, therefore, that here again the active wavelengths are those in the visible spectrum. This is supported by preliminary experiments in which milk in a number of glass vessels of the same size but covered with 'Cellophane' of different colours was exposed to sunlight. The destructive power of the transmitted light increased in the order: red (low activity), yellow, green, blue, the blue light being only a little less active than the full sunlight (through glass). Sunlight falling directly on the milk was rather more active in destroying the lipase than sunlight through glass.

If oxygen is removed as thoroughly as possible from milk by boiling it under reduced pressure or by washing oxygen out with a stream of pure nitrogen, the destructive effect of subsequent exposure of the milk to sunlight is greatly diminished. The addition of 0·1 per cent of sodium cyanide also protects (though not completely) the lipase from destruction by direct sunlight. On the other hand, the rate of photo-destruction of the enzyme may be markedly increased by adding 100 μgm. of riboflavin to 100 ml. of the milk (cow's milk normally contains some 150 μgm. of riboflavin per 100 ml.). This recalls the similar sensitizing effect of riboflavin on the photo-oxidation of vitamin C[3]. If milk is exposed for a few minutes to sunlight and then kept in the dark, the destruction of lipase continues rapidly at first but ceases in about three hours. Nevertheless, fresh milk, added to milk in which the lipase has been completely inactivated by light, loses none of its activity even on standing for several hours. The investigation is being extended.

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Shinfield, Reading. Jan. 8.

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