LETTERS TO THE EDITORS

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Application of the Chemiluminescence Test for Hæmatin to Plant Tissues

CYTOCHROMES and other catalysts capable of yielding hæmatin are widely distributed in living tissues. It is, therefore, possible *a priori* that many tissues will give a positive reaction in the luminescence test with *o*-amino phthalic acid cyclic hydrazide ('luminol') when treated in some way which will lead to the formation of hæmatin. However, in the forensic application of the test it has generally been found that vegetable matter, either fresh or rotting, gives a negative reaction[1].

The confirmation by Keilin and Wang[2] of the hæmoprotein nature of the red pigment of the root nodules of leguminous plants, and their identification of this pigment as a hæmoglobin, prompted an examination of the chemiluminescence reaction of nodular tissue.

The reagent was made by dissolving 100 mgm. luminol and 5·0 gm. Na_2CO_3 in 100 ml. water and adding 20 ml. H_2O_2 (10 vol. approx.) just before use. The very faint inherent luminescence of this solution was not regarded as sufficient to interfere with the reactions now described.

Solutions for test were then prepared as follows: (*A*) 4 ml. water + 1 ml. 5 per cent NaOH. (*B*) 50 mgm. fresh root nodules (*Trifolium hybridum*) ground with 1 ml. 5 per cent NaOH and diluted with 4 ml. water. (*C*) 50 mgm. fresh leaf tissue, from the same plant, ground with 1 ml. 5 per cent NaOH and diluted with 4 ml. water. (*D*) 50 mgm. fresh nodules, also from the same plant, dried in the steam oven and then extracted by grinding with 5 ml. water.

3 ml. portions of these test solutions were then added in the dark to 5 ml. portions of the reagent in test tubes. The reactions were as follows: (*A*) No reaction. On adding extra NaOH solution, no change. (*B*) An intense and vivid whitish-blue luminescence. This faded gradually, but addition of extra alkali gave a perceptible brightening of the fading luminescence. (*C*) A faint increase over the inherent luminosity of the reagent, the colour being altered somewhat by the presence of chlorophyll derivatives. Little, if any, change on adding extra alkali. (*D*) A strongly positive reaction, similar to that given by solution (*B*) but not nearly so intense. On adding extra 5 per cent NaOH to the fading solution, the luminescence increased so as to become much stronger than initially. Fading did not seem so rapid as in the case of solution (*B*).

The observations were then extended to solutions of (*E*) 50 mgm. stem tissue and (*F*) 50 mgm. root tissue, also from *T. hybridum*, and of (*G*) 50 mgm. potato tuber, each being ground with 1 ml. 5 per cent NaOH and diluted with 4 ml. water. These solutions reacted to the test in the same way as the leaf tissue solution (*C*), except that secondary addition of alkali decreased luminescence. A water extract of fresh nodules (*H*) behaves in the same way as the water extract of dried nodules (*D*).

These observations are consistent with the hypothesis that the most powerfully reacting compound in the plant tissues examined is the hæmoglobin of the root nodules, and that other compounds capable of yielding hæmatin are present in concentrations too minute to afford amounts of hæmatin capable of giving a strong reaction under the specified conditions. The results of tests (*D*) and (*H*) are consistent with the hypothesis that hæmatin formation during grinding with water or drying is too small to give a really intense reaction, but that subsequent addition of alkali splits off the prosthetic group of the hæmoglobin from the protein moiety and leads to the production of hæmatin so as to give a stronger reaction than initially.

It is possible that the chemiluminescence test may be of use as a rapid sorting test for locating in plant tissues unusual concentrations of substances capable of yielding hæmatin, or for the demonstration of hæmoglobin in leguminous root nodules where adequate spectroscopic equipment is not available.

I wish to thank Prof. D. C. Harrison for his advice on various possible interpretations of the reaction.

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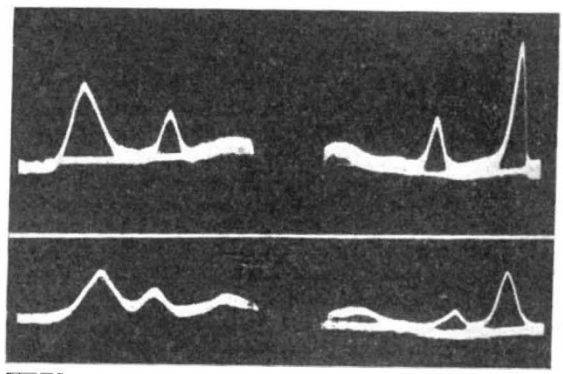
[1] McGrath, J., *Brit. Med. J.*, 156 (1942).
[2] Keilin, D., and Wang, Y. L., *Nature*, 155, 227 (1945).

Electrophoretic and Enzymatic Fractionation of Casein from Human Milk

SOME years ago I examined electrophoretically casein from cow's milk. It was established that this protein is not homogeneous[1]. The electrophoretic diagram showed three different boundaries which were assumed to correspond to three casein fractions, α-, β- and γ-casein. The fastest moving of these, the α-fraction, was isolated, and on analysis its nitrogen/phosphorus ratio was found to be higher than that of total casein.

It has been considered worth while, especially in view of the importance of the organically bound phosphorus for breast-fed babies, also to examine *human* milk casein for the existence of electrophoretically separable fractions and their phosphorus content.

As may be seen from the accompanying record, the electrophoretic pattern of human milk casein is similar to that of casein from cow's milk. Three



Descending ← → Ascending

ELECTROPHORETIC PATTERNS OF CASEIN FROM COW'S MILK (ABOVE) AND HUMAN MILK (BELOW); *p*H 7·62 (PHOSPHATE BUFFER), IONIC STRENGTH 0·15 (PHOSPHATE 0·10 + SODIUM CHLORIDE 0·05). THE PHOTOGRAPHS WERE TAKEN AFTER MIGRATION FOR 105 MIN. (ABOVE) AND 165 MIN. (BELOW) AT 5·70 VOLTS/CM. PROTEIN CONCENTRATION 1 PER CENT.