

It is hoped to complete detailed investigations on the spread and effect of the disease and on the nature of the vector in the course of the present growing season.

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¹ Caldwell, J., *Ann. App. Biol.*, **21**, 191 (1934).

Effect of Copper-Enzyme Poisons on Soil Nitrification

THE possible biological importance of an activated copper nitrogen complex has been repeatedly stressed by Baudisch¹. I have therefore tried the effect of some well-known copper-enzyme poisons on soil nitrification, which is the process whereby ammonium ions are oxidized to nitrite ions and thence to nitrate ions by the microflora in soil. The percolation apparatus used in this work was an improved and simplified version² of the one already described³.

Ten grams of a Kent marsh soil were initially percolated with 100 ml. of *M*/200 ammonium chloride to stimulate nitrifying activity in the soil. When the soil was nitrifying well (as indicated by a rapid rise of nitrate in the percolate) the percolate was discarded and the soil rinsed three times with 50 ml. lots of a *M*/250 solution of one of the poisons. After the poison had been in contact with the soil for two hours, excess was washed out with 3 × 50 ml. lots of distilled water and the soil re-percolated overnight either with 100 ml. distilled water or with 100 ml. of a *M*/1,000 solution of cupric, ferrous or manganous sulphate. The next morning this new percolate was again discarded and replaced by 100 ml. of *M*/200 ammonium chloride. The nitrite-nitrogen plus nitrate-nitrogen concentration in the percolate was thereafter estimated daily by phenoldisulphonic acid and the amount of nitrite-nitrogen plus nitrate-nitrogen formed per gram of soil calculated from the results.

The results from a number of different experiments show that all the four poisons tried reduced the rate of nitrification in soil. There is furthermore evidence that cupric, and perhaps ferrous, ions are capable of partially reversing the poisoning effect.

MICROGRAMS OF NITRITE PLUS NITRATE-NITROGEN FORMED IN TWO DAYS PER GRAM OF KENT MARSH SOIL

Poisoned with	Perfused overnight with			
	Water	CuSO ₄	FeSO ₄	MnSO ₄
<i>M</i> /250 potassium ethyl xanthate	tr.	120	50	70
<i>M</i> /250 sodium diethyl dithiocarbamate	20	220	50	20
<i>M</i> /250 salicylaldehyde	10	180	120	0
<i>M</i> /250 allylthiourea	50	50	50	50
Unpoisoned control	400	*	*	*

* A separate set of control experiments showed that the metal solutions themselves had no effect on the nitrification rate.

Experiments in which the oxidation of nitrite to nitrate by soil was studied separately gave results that showed a similar action of copper poisons on this process. The effect here was not, however, quite so strong.

The results suggest that copper and/or some allied elements play an important part in the oxidation of ammonium ions in soil by the soil microflora. Preliminary results obtained by Drs. Mann and Heinze in this laboratory show that the rate of oxidation of manganous ions may also be reduced by these same copper-enzyme poisons. Reactivation is, however, difficult here because of the toxic action of quite dilute copper solutions on manganese oxidation.

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¹ See, for example, Baudisch, O., *Soil Science*, **60**, 173 (1945).

² Lees, H., in the press.

³ Lees, H., and Quastel, J. H., *Chem. and Ind.*, 238 (1944).

Anti-Oxygen Stabilization of Bilirubin in Alkaline Medium by Ascorbic Acid and Cysteine

WE have shown in earlier publications^{1,2} that ascorbic acid and cysteine prevent the oxidation of bilirubin in alkaline medium. The effect of both substances was ascribed hypothetically to their anti-oxygen properties. The mechanism of action of the anti-oxygen agents had not been elucidated. On the other hand, the protective action of ascorbic acid and cysteine for bilirubin might equally be explained as a reducing one. If such a view is valid, one would expect oxidized bilirubin to be promptly reduced in alkaline solution, when vitamin C or cysteine is added.

This hypothesis has been verified as follows: 10 mgm. bilirubin Hoffmann-La Roche, identical with Fischer's product, are dissolved in 500 c.c. *N*/50 sodium hydroxide. The oxidation of the pigment, which begins almost immediately, is characterized photometrically, as in previous experiments in collaboration with A. Lambrechts³, by a continuous decrease of absorption in the region of 4300 Å. 24 hours later, while the initial extinction coefficient at 4300 Å. is 1.84 for a stabilized bilirubin solution, the oxidized bilirubin solution, on the contrary, shows a considerable decrease in its absorption, its

extinction coefficient being 0.22. To two 100 c.c. samples of this oxidized bilirubin solution, we then add respectively 40 mgm. ascorbic acid and 94 mgm. cysteine hydrochloride neutralized by 10 per cent sodium hydroxide. These solutions are immediately examined by means of the Pulfrich photometer, and again after 3 and 6 hours. The accompanying table shows the photometric values recorded.

S.	Oxidized bilirubin + ascorbic acid			Oxidized bilirubin + cysteine		
	K immed.	K after 3 hr.	K after 6 hr.	K immed.	K after 3 hr.	K after 6 hr.
43	0.22	0.22	0.23	0.22	0.22	0.21
45	0.12	0.12	0.13	0.14	0.12	0.11
47	0.04	0.04	0.05	0.05	0.03	0.03
50-75	0.00	0.00	0.00	0.00	0.00	0.00

Conclusions. Ascorbic acid and cysteine do not reduce oxidized bilirubin in alkaline medium. It rather seems that both substances prevent directly the oxidation of bilirubin. In collaboration with R. Roseman⁴, similar observations were made with some polyphenols easily oxidized in alkaline solutions. The experiments are being continued.

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¹ Barac, G., *Bull. Soc. Chim. Biol.*, **21**, 1163 (1939).

² Barac, G., *C.R. Soc. Biol.*, in the press.

³ Lambrechts, A., and Barac, G., *Bull. Soc. Chim. Biol.*, **21**, 1171 (1939).

⁴ Barac, G., and Roseman, R., *Bull. Soc. Chim. Biol.*, in the press.

Colour Receptors of the Human Fovea

AS soon as the results of Granit's micro-electrode experiments on the retinae of animals were published, it was clear that a method was wanted for obtaining similar information with regard to the colour vision of man. This led to the development of the retinal micro-stimulator, which consists essentially of a microscope used in reverse, so that greatly diminished images of suitable test light-sources are presented to the eyes of the observer.

With apparatus of suitable design, it is possible to test, point by point, the colour vision of a chosen area of the retina. The dimensions I have used are such that each centimetre on the plotting-board of the apparatus corresponds with 'the cone intercentre distance', that is, the distance between the centre of one foveal cone and that of its next-door neighbour. It has been found possible to record the positions of the test light-sources with an accuracy corresponding to one tenth of this distance. A number of experiments have been performed with this technique, but those to be reported here concern the theories of colour vision. As is well known, Thomas Young's trichromatic theory postulated three colour sensations: red, green and blue. Granit, on the other hand, found in the retinae of several types of mammals one 'dominator' and seven 'modulators'. The former was a sense-organ which responded to stimulation by light coming from most of the visible spectrum. The latter, on the contrary, were receptors with responses limited to a narrow part of the spectrum only. Granit found 'modulators' with maxima at the following wavelengths in Angströms: 6000, 5800; 5400, 5200, 5000; 4600 and 4400. Thus, whereas the difference between two neighbouring units was usually two hundred Angströms, in two places the difference was double that amount, hence dividing them into three groups: yellow-orange, green and blue-violet.

Granit's conclusion was that each of the hypothetical 'sensations' of Thomas Young consisted of two or more kinds of 'modulator'. It should be pointed out, however, that whereas Granit's work has been performed on animals, Thomas Young's theory was intended to apply only to man. Physiologists are rightly cautious in such a case as this, for what is found with the former may differ widely from what is found with the latter.

The following results have been obtained. When white light from a small metal filament electric lamp is caused to move slowly over the fovea, as a narrow exploring pencil, in some places it appears red, in other places green, and in still other places blue. When red, it matches in colour a pencil of red light of 6400 Å.; when green, it matches a pencil of green 5400 Å.; and when blue, it matches blue of 4800 Å. The precise position in the fovea of some of these specific points has been determined with reference to the point of fixation, by measuring the distance between the white test-light and the monochromatic light on which the gaze is fixed. Between these foveal points with specific colour responses are numerous other points having a non-specific response which may be either white or yellow.

A monochromatic orange light of 6200 Å. is seen as red in some foveal positions, and as pale orange in others. Sometimes a minute black spot, due to the presence of an unstimulated receptor, is perceived. A monochromatic yellow light of 5800 Å. behaves like a white light, in sometimes appearing white (or pale yellow), sometimes red, sometimes green and sometimes orange. A monochromatic green light of 5400 Å. sometimes appears green, and sometimes very pale green or even white, as it is moved slowly from place to place over the fovea.

These experiments point to the following conclusions.

(1) Thomas Young's trichromatic theory of colour vision is substantially correct, since the above tests are held to prove the existence of red receptors, green receptors and blue receptors, in the human fovea.