$1.7 \cdot 10^{-6}$ mg/mg of preparation, acetylcholine caused a slight slowing down of the heart action, but no inotropic effect was observed. An injection of $2.6 \cdot 10^{-6}$ mg/mg of weight was followed by a pronounced slowing down of the heart rate and a decrease of the amplitude (Figure 2). The return to normal, however, was quicker than after adrenalin. Overdosing resulted, as with adrenalin, in stopping the heart action.

Any comparison of the above results with those for other invertebrates is difficult because of the difference in the methods used. Certain analogies, however, may be found between the effect of acetylcholine in spiders and that in molluscs[2] and lower crustaceans[3]. The effect of adrenalin, on the other hand, is similar with regard to the chronotropic effect, but the reverse in the inotropic effect.

Résumé. Les auteurs ont étudié l'effet de l'adrénaline et de l'acétylocholine sur le rythme du cœur chez l'araignée *Tegenaria atrica* C. L. Koch. L'enregistrement s'est fait au moyen de l'électroencephalographe. L'injection de l'adrénaline provoquait l'accélération du rythme du cœur, l'acétylocholine – son ralentissement.

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Autotrophic Incorporation of $C^{14}O_2$ in *Cuscuta australis* in Relation to its Parasitism

Introduction. Although *Cuscuta* is traditionally regarded as a classic example of parasite plant, unable to grow autotrophically, several reports in the literature of less recent years suggest the possibility of dodder photosynthesis. Some of these concern the presence of chlorophyll[1], others the light-induced oxygen evolution[2]. More recent references concern the possibility of *Cuscuta* growing and synthesizing starch in vitro culture without an external sugar supply[3,4] and this is difficult to explain from a point of view of a total heterotrophy.

Recently[5] it has been found that $C^{14}O_2$ fixation in two species of *Cuscuta* is a photosynthetic process and virtually all the radioactivity is present in the sucrose zone. On the contrary, CIFERRI[6,7] reported that *C. epythymum* could really carry out only a 'heterotrophic fixation' through the carboxylation of phosphoenolpyruvic acid.

In order to reinvestigate the whole problem of the photosynthetic activity of *Cuscuta*, we have examined both the pigment composition of this plant[8,9] and the light-driven $C^{14}O_2$ incorporation into organic compounds.

This report deals with this latter topic.

Methods. Seedlings of *C. australis* were utilized, which were grown for 6 days in continuous light at $25 \mp 1°C$ and stems of the same dodder detached from the host (*Medicago sativa*) at various stages of growth. Before the exposure to $C^{14}O_2$, the seedlings were kept 12 h in the dark and 15 min in the light, the stems were kept on moistened filter paper for 6 h in the dark and 15 min in the light. Then seedlings or stems were placed in plexiglass containers (250 ml) and exposed to an atmosphere containing $C^{14}O_2$ (30 μC $Na_2C^{14}O_3$, sp. act. 1.16 mC/mM, together with sufficient carrier to give a final atmosphere of 2% CO_2 within the box). After 1 h CO_2 was evacuated from the chambers and the plants homogenized twice more in boiling 80% ethanol. The combined alcoholic extracts were evaporated to dryness under reduced pressure at 40°C. The residue was extracted with ethyl ether and ethanol-ether (3:1), dissolved in distilled water, and again evaporated. Then it was dissolved in glycine buffer pH 8.5 and incubated for 2 h at 37°C with alkaline phosphatase and Mg^{++} ($5 \cdot 10^{-4} M$).

The water-soluble material was fractionated with Dowex resins in $1 \cdot 6$ cm columns. The basic fraction (amino acids) was eluted from Dowex 50W · 8 (100–200 mesh) (H^+) with 0.25N NaOH, and the acidic fraction (organic acids) from Dowex $1 \cdot 10$ (200–400 mesh) (formate form) with 8N formic acid.

The residue from alcohol extraction was hydrolysed for 3 h in 10 ml of 1N H_2SO_4 in boiling water. It was neutralized with BaOH, filtered, and the excess of BaOH precipitated. The same methods were applied to the hydrolysed as to the water-soluble material.

A sample of all the fractions was evaporated to dryness on a planchette and analysed for radioactivity on a windowless gas-flow counter.

Aliquots of the neutral fractions of the ethanol soluble extract were applied to Whatman No. 1 filter paper and chromatographed using the upper layer of a mixture of ethylacetate-acetic acid-water (3:1:3)[10]. Known components in concentrations of 25–50 μg were simultaneously chromatographed. Sheets of X-ray film, $40 \cdot 25$ cm, were exposed to the chromatographs and subsequently developed in the usual manner. Spots on the radiochromatographs were identified by means of specific spray reagents, comparison of Rf values with those of standards and cochromatography.

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The distribution of C^{14} incorporated in 1 g (wet weight) of seedlings, stems, and flowering buds of *C. australis* during 1 h

	Seedlings					Stems					Flowers				
	Light (L) cpm	%	Dark (D) cpm	%	Lcpm/ Dcpm	Light (L) cpm	%	Dark (D) cpm	%	Lcpm/ Dcpm	Light (L) cpm	%	Dark (D) cpm	%	Lcpm/ Dcpm
Ethanol-soluble fractions															
Neutral	54,127	*47*	765	*1.9*	70.65	29,223	*27.3*	226	*0.6*	129.3	75,014	*36.3*	386	*1.5*	194.33
Basic	25,821	*22.4*	11,215	*28.4*	2.3	22,920	*21.4*	10,498	*29*	2.18	48,767	*23.6*	10,395	*39.6*	4.69
Acidic	25,389	*22*	24,352	*61.7*	1.04	44,958	*42.1*	23,918	*66.3*	1.87	67,959	*32.9*	12,994	*49.5*	5.23
Ether extract	803	*0.7*	353	*0.9*	2.27	3,927	*3.7*	202	*0.5*	19.44	3,266	*1.6*	345	*1.3*	9.46
Total	106,140		36,685		2.9	101,028		34,844		2.9	195,006		24,120		8
Ethanol-insoluble fractions															
Neutral	6,396	*5.5*	1,298	*3.3*	4.92	1,677	*1.6*	86	*0.2*	19.5	7,593	*3.6*	45	*0.2*	168.7
Acidic and basic	2,620	*2.3*	1,483	*3.7*	1.76	4,118	*3.8*	1,145	*3.2*	3.59	4,146	*2*	2,062	*7.8*	2.01
Total	9,016		2,781		3.24	5,795		1,231		4.7	11,739		2,107		5.6

Results and conclusions. The total amount of C^{14} (in counts/min) and the distribution of radioactivity incorporated into the various fractions by seedlings, stems, and flowers in the light and in the dark are shown in the Table. From this it is evident that the light greatly stimulates the assimilation of $C^{14}O_2$: the ratio Lcpm/Dcpm among the total quantities is about 3 in seedlings and stems and notably increases in the flowering buds, probably owing to the greater quantity of chlorophyll present in these materials (108 γ/g WW). Most of the activity is incorporated into the ethanol-soluble fractions both in the light and in the dark (more than 92% of the total radioactivity).

In the light the neutral fraction (sugars) is heavily labelled while in the dark very little radioactivity appears in this fraction. The ratio Lcpm/Dcpm between these fractions is 70 in the seedlings and increases to 129 in the stems and 200 in the flowers.

The incorporation into the amino acids and the organic acids appears to be slightly stimulated by the light, the ratios Lcpm/Dcpm being of 2 and 1 respectively in seedlings and stems and 4–5 in flowers. In the dark the amino acids and the organic acids are the major compounds present, according to the dark fixation in the non-parasiting plants.

In the light and in the dark, less than 7–8% of the total radioactivity was found in the ethanol insoluble fractions: the ratio Lcpm/Dcpm between the neutral fractions is 5 in the seedlings and increases to 20 and 168 in stems and flowers respectively.

The separation of the neutral fraction by one-dimensional chromatography shows that the greatest part of radioactivity corresponds to the sucrose, glucose and fructose regions; the most conspicuous peak of radioactivity is in the sucrose zone.

From the results described above it is clear that light remarkably stimulates $C^{14}O_2$ fixation in all the stages of *C. australis* life (seedlings, stems and flowers) and such influence appears much more evident when we consider the neutral fractions, whether of the water-soluble extract or of the water-insoluble. The complete analysis of our results shows also a remarkable increase of metabolic rate of the *Cuscuta* stems in respect to the seedlings. In fact in the stems the increase in percentage of the organic acids in the light is probably due to a faster metabolism; while the ratio Lcpm/Dcpm of the neutral fractions increases to 129, that of the organic acids is always less than two. This shows that the light stimulates the synthesis of these substances not directly but rather indirectly via phosphoglyceric acid-phosphoenolpyruvic acid and Krebs pathway. Also the increase of C^{14} incorporation into the neutral fractions of the water-insoluble extract is a sign of a much faster metabolism which is responsible for an accumulation of polysaccharides. The same considerations are also valid for flowering buds.

In our experimental conditions CO_2 fixation seems to be of an autotrophic type and our results reveal a more thorough analysis than the data reported by MacLeod[5]. The comparison becomes more difficult when compared with Ciferri's negative results[6,7], which are not wholly justified even by his experimental data.

Cuscuta by itself then, not only does not require amino nitrogen for life and growth[3,4,11], but finds itself in a potential autotrophic condition. Although the relationship between the various groups of pigments are of the same order as in the non-parasite plants[9] and the CO_2 fixation is of the photosynthetic type, probably the smaller quantity of chlorophyll and the reduced surface of chlorenchyma act as factors limiting photosynthesis in such a way that the intensity of photosynthesis is no longer similar to normal plants. The autotrophic survival of the seedlings in nature is perhaps impeded by these characteristics which, at this stage of plant life, are more relevant and are in addition to slower metabolism.

C. australis is unable to survive in the ground if it has not the possibility of becoming parasitic to other plants; as a parasite it retains its faculty of photosynthesis, as is clearly shown by our experiments, but this possibility remains perhaps at its potential stage, when the competition in CO_2 utilization is added to the disposal of large quantities of carbohydrates by the parasitized plants.

Riassunto. L'incorporazione della $C^{14}O_2$ in *C. australis* appare chiaramente di tipo fotosintetico in qualunque stadio di vita della pianta (plantula, fusto, infiorescenza). Fusti ed infiorescenze appaiono dotati di un metabolismo più veloce rispetto alle plantule.

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