

a quantum by the quinoline group of the quinine molecule. Vibrational energy, transferred to the secondary hydroxyl group adjacent, may increase the reactivity of the latter.

Parallel experiments with quinine derivatives are being made. Temperature coefficients and the effects of variable dichromate concentrations, especially at low acidities, are being investigated.

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[CONTRIBUTION FROM THE LABORATORY OF PLANT NUTRITION OF THE UNIVERSITY OF CALIFORNIA]

## ATTEMPTED PHOTOSYNTHESIS OF CARBOHYDRATES IN VITRO WITH VISIBLE LIGHT

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The appearance in 1927 of three papers<sup>1</sup> by E. C. C. Baly and others on the "Photosynthesis of Naturally Occurring Compounds" was of very great interest to those concerned with photosynthesis as it occurs in the living green plant. Particular importance was attached to the second paper of this series, in which was described a method for the photosynthesis of carbohydrates from carbonic acid by means of visible light and colored solid catalysts. Forty futile attempts were made by the writer to repeat the experiments of Baly with visible light and nickel and cobalt carbonates as catalysts, with hopes of extending these experiments. Special precautions were taken to eliminate contamination of the photosynthetic system by impurities from various sources, precautions which were more rigorous than those described by Baly in the second paper mentioned above.

In paper II, Baly describes a precipitation method for preparation of carbonates of nickel and cobalt by adding cold solutions of nickel or cobalt nitrate and of potassium carbonate together and washing the precipitate with hot water to remove all foreign ions and alkali. The precipitate is then dried at 100° and powdered. The powder requires activation before introduction into the photosynthetic chamber, this activation being accomplished either by heating at 120–140° for thirty minutes or by exposing the powder to ultraviolet light for thirty minutes. The powder is then placed in conductivity water through which a stream of carbon dioxide is bubbled, this system being illuminated by visible light from electric lamps. After two hours, the mixture is filtered and the filtrate evaporated. The final residue is extracted with methanol, which removes the photosynthesized substance. This substance is said to reduce Benedict's solution, as well as to give other tests for carbohydrates. Baly's directions are rather incomplete as to details but they were followed as closely as possible, with some refinements, in the experiments described below.

An effort was made to exclude all sources of contamination, both organic and in-

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<sup>1</sup> E. C. C. Baly, J. B. Davies, M. R. Johnson and H. Shanassy, *Proc. Roy. Soc. (London)*, **A116**, 197 (1927); E. C. C. Baly, W. E. Stephen and N. R. Hood, *ibid.*, **A116**, 212 (1927); E. C. C. Baly and J. B. Davies, *ibid.*, **A116**, 219 (1927).

organic, in the preparation of the materials to be used in the system. All the water was redistilled in an all-glass Pyrex still, being collected and kept in Pyrex glass-stoppered flasks or flasks whose corks were covered with tin foil. In many cases the water was saturated previous to the experiment with the bicarbonate of the metal involved by bubbling carbon dioxide from the purification train through the water and carbonate mixtures for one and a half to two hours and filtering off excess carbonate. Using such water, the surface of the activated catalyst should not dissolve off immediately when placed in the illumination chambers. The resulting solution, tested with brom thymol blue, had a  $P_{\text{H}}$  of 6.6–6.9 in the case of nickel carbonate and 6.2–6.3 with cobalt carbonate, so that alkalinity, warned against by Baly, was avoided.

The carbon dioxide was supplied from a tank, having been originally made by heating calcium carbonate. However, such carbon dioxide contains impurities, as is shown by the yellow oil which is left on sublimation of solid carbon dioxide. To purify it, the carbon dioxide was led from the tank through a lead tube, soldered to the valve outlet, into a Pyrex combustion tube which lay in a gas furnace. The juncture between the combustion tube and the lead tube was sealed with Insalute cement. This combustion tube, of 2.5 cm. external diameter, was heated to red heat. The entering gas first passed through a 60-cm. column of cupric oxide granules, and then over a reduced copper spiral 13 cm. long. After issuing from the combustion tube, it was washed through about 300 cc. of water and then passed through a cooling coil, made of Pyrex tubing of 0.9 cm. external diameter, containing 15 coils, each having a 4 cm. internal diameter. The object of this cooling was to freeze out any impurities that would not be volatile at the temperature of solid carbon dioxide, with which the cooling coil was surrounded. From this coil, the carbon dioxide entered the illuminated reaction vessels. Three Pyrex tubes were used as reaction chambers. Their wall thickness was 2 mm., their inside diameter 2.6 cm. and their illuminated length 26 cm. The three tubes presented an area of 203 sq. cm. to the source of light, and had a total volume of 450 cc. They were provided with ground-glass stoppers. The carbon dioxide bubbled through each tube in succession, at the rate of 12 liters per hour, the bubbles keeping the catalyst powder continually stirred and in suspension. Throughout the entire apparatus, there were no corks or rubber tubing. From the time the carbon dioxide entered the hot combustion tube until it had passed into the air from the last of the illuminated chambers, it was in an all-glass enclosing system. Before each experiment, the glass outlet from the purification train was sealed to the glass tube which entered the reaction vessels.

A number of catalysts were used, chiefly the carbonates of nickel and cobalt. The catalysts most used were prepared by adding one mole of the nitrate of nickel or cobalt (Baker's c. p.) dissolved in 150 cc. of water to one mole of sodium carbonate in 450 cc. of water, each solution having first been filtered. The precipitate was added to 550 cc. of water, heated to boiling and centrifuged, the liquid being discarded and the precipitate washed again in the same manner. The nickel carbonate was washed nineteen times and the cobalt carbonate fourteen times. They then contained no nitrate, as shown by the ferrous sulfate ring test, and less than 0.0007% of chloride and 0.0002% of sulfate as shown by tests with silver nitrate and barium chloride. After drying for two and a half hours at 100°, the carbonates were ground to a fine powder in an agate mortar. Baker's c. p. nickel carbonate and cobalt carbonate were washed twelve times with boiling water, 100 g. to 550 cc. of water, which was removed by centrifugation. They then contained less than 0.0007% of chloride and 0.0002% of sulfate, which were the limits of the tests used. Some other catalysts tried were:  $\text{Co}_3\text{O}_4$  deposited on pumice; charcoal made from sucrose; the oxides of nickel and cobalt made by heating the carbonates; ferric oxide dialyzed with collodion; ferric hydroxide made by hydrolysis of ferric chloride, and dialysis with collodion; and colloidal cupric oxide and platinum, made by under-

The catalysts were activated chiefly by exposure to the radiation from an air-cooled, Hanovia, mercury arc lamp at a distance of 15 cm., the powder being spread on a 25-cm. watch glass and stirred every five minutes during the activation period of thirty minutes. Heating to 120–130° in an oven was also tried as a means of activation. The time between activation of the powder and exposure to carbon dioxide and light was from eight to ten minutes; 1.5 g. of powder was used for each illuminated tube, or 4.5 g. for 450 cc. of water. Most of the exposures to light lasted for one and a half hours, but some experiments lasted two, three and six and a half hours.

Various light intensities were used, a conical reflector being placed behind the Mazda lights. The wattages of the lamps were 100, 300, 500 and 1000, the 100 and 300 watt lamps being used most. The distance of the filament from the illumination chambers was from 7.6 to 15 cm. In two experiments with nickel and cobalt carbonates, bright sunlight was used as a light source, carbon dioxide being taken directly from the tank through a rubber hose in these cases only.

In most of the experiments a stream of air from an electric fan was kept continually flowing over the illumination chambers to prevent excessive heating from the light source. The temperatures of the suspension in the tube at the end of the illumination period with various light intensities were as follows: 100 watt, 25°; 300 watt, 33°; 500 watt, 36°; 1000 watt, 52°; 300 watt, no fan, 57°; and sunlight, 35°.

After the illumination period, the aqueous suspensions from all these tubes were filtered and the filtrate evaporated to 10 cc. on the steam-bath. The carbonate was filtered off and the filtrate treated with a few drops of hydrochloric acid, and heated on a water-bath for one hour to hydrolyze any complex carbohydrates that might have been synthesized. After neutralization with sodium carbonate, the solution was evaporated to 2 cc., added to 5 cc. of Benedict's solution and heated to boiling. The solution was then centrifuged for ten minutes to deposit any cuprous oxide that might not have been visible without centrifugation. One milligram of glucose gives a quite large precipitate of cuprous oxide under these conditions, and probably no difficulty would be found in detecting 0.1 of a milligram of glucose by this method.

The carbon dioxide was, in several experiments, bubbled through 300 cc. of sodium bicarbonate solution mixed with glass beads, after coming from the combustion tube. The gas issuing from such a system in fifty minutes gave 19 cc. of gas which was not absorbed by a solution of sodium hydroxide, showing the presence of a gaseous impurity. Other experiments were run in which the tank carbon dioxide and the combustion furnace were not used, but carbon dioxide was obtained by heating pure sodium bicarbonate. Here, again, all-glass connections were used and the gas was passed through a solution of sodium bicarbonate and the cooling coil before entering the illumination chamber.

Comparing, as nearly as possible, the above conditions with the suspension's surface exposed to light and amount of catalyst used in Baly's experiments, a yield of about 10 mg. of carbohydrates should have been obtained. The test used would detect one hundredth of this amount. At the time this work was done, the paper<sup>2</sup> on the temperature coefficient of the reaction had not appeared. Baly and Hood describe a maximum yield at 31°. The work described above for 100 and 300 watt lamps should have given satisfactory results so far as temperature is concerned.

However, in no case was a cuprous oxide precipitate found in Benedict's test, all the experiments giving totally negative results. In such work there are many sources of error. The writer does not consider that Baly has necessarily removed all sources of organic impurities, since he used carbon dioxide from a cylinder without purification. His control experi-

<sup>2</sup> E. C. C. Baly and N. R. Hood, *Proc. Roy. Soc. (London)*, **A122**, 393 (1929).

ments do not necessarily prove the total absence of organic impurities in his system, since it is possible that his carbon dioxide might have contained an organic impurity which required all of the conditions of his successful experiments for its transformation to an organic substance giving the tests described above. Such an impurity might not have been detected in his control experiments.

The recent papers<sup>3</sup> of Rao and Dhar report success in the synthesis of carbohydrates from carbon dioxide by means of the carbonates of nickel and cobalt in sunlight. The data presented are not quantitative. It is interesting that they obtained carbohydrates in 2% sodium bicarbonate solution, while Baly reported that all traces of alkali must be removed from the catalyst in order to obtain positive results.

Emerson,<sup>4</sup> using a carbonate of nickel prepared electrolytically and exposed to visible light before use, was not able to detect any production of oxygen as a result of illuminating the system, nor did he find that the absorption of carbon dioxide (found to be reversible at any point) was changed in rate by illumination. He also found no carbohydrate by the test of visible charring.

In paper IV, and in *Nature*,<sup>5</sup> Baly describes other methods of preparation of different and more active catalysts. It is quite possible that the method of catalyst preparation used by the writer is not exactly the same as Baly's, but so far as Baly's published directions permit, the author has been unable to reproduce, with positive results, any of Baly's experiments with visible light, using nickel or cobalt carbonates prepared by the precipitation method. In the opinion of the writer, none of these papers describe in sufficient detail the methods used, so that Baly's experiments might readily be successfully repeated from his directions.

The writer wishes to thank Professor D. R. Hoagland for certain suggestions received during the progress of the work.

### Summary

1. Attempts were made to repeat the experiments reported by Baly in 1927, in which he claims to have synthesized carbohydrates *in vitro* from carbon dioxide and water, using the carbonates of nickel and cobalt as catalysts, suspended in water saturated with carbon dioxide and illuminated by visible light.

2. Special precautions were taken to purify the carbon dioxide used and Benedict's test was used for the detection of carbohydrates.

3. The results were all absolutely negative.

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<sup>3</sup> G. G. Rao and N. R. Dhar, *J. Phys. Chem.*, **35**, 1418, 1424 (1931).

<sup>4</sup> R. Emerson, *J. Gen. Physiol.*, **13**, 163 (1929).

<sup>5</sup> E. C. C. Baly, *Nature*, **126**, No. 3182, 666 (1930).