The presence of an acting peripheral mechanism in cardiectomised animals suggests the following possibilities:—

1. That the peripheral mechanism is active to some small degree in all parts of the normal body; it is, perhaps, this mechanism which favours local action of substances. 2. That this mechanism may take an active share in the process of distribution in organs which are normally deficient in circulation. The brain, for instance, has no lymphatics, and the exchange of fluid material with the blood capillaries is said to be there somewhat deficient. 3. That the peripheral mechanism gets into prominence in pathological conditions in which there is either a local or general deficiency of the cardio-vascular circulation.

The Mechanism of Carbon Assimilation: Part III.

By Francis L. Usher and J. H. Priestley.

(Communicated by Dr. M. W. Travers, F.R.S. Received April 13,—Read June 1, 1911.)

Some experiments and conclusions recorded in two papers* published in 1906 have been subjected to criticism by several investigators, and the present paper has been written with the object of presenting some new facts bearing on the problem of carbon assimilation, which incidentally support some of those conclusions. We also take this opportunity to restate the theory originally advanced, with such modifications as may be necessary, and to reply to a few of the more important objections to it which have been raised.

The observations recorded below are concerned only with the initial stages of the photosynthetic process, that is to say, with the formation of the primary photolytic products from carbon dioxide, and with the evolution of oxygen. In the papers referred to some evidence was given in support of the belief that aqueous carbon dioxide is decomposed by light under the conditions obtaining in a green leaf, the immediate products of this decomposition being hydrogen peroxide and formaldehyde; and it is easy to see that the production of these two substances would satisfactorily account both for the oxygen and the carbohydrate, which are the first visible results of the natural process. As the evidence put forward was to some extent indirect,

* 'Roy. Soc. Proc.,' B, vol. 77, p. 369; and vol. 78, p. 318.

wholly so in the case of hydrogen peroxide, it was thought advisable to supplement it by further experiments.

1. *The Products of Photolysis of Aqueous Carbon Dioxide.*

(a) *In Vitro.*—No further experiments have been made with solutions of uranium salts; either no sensitiser at all, or chlorophyll films, as described in Part II, have been employed. It has been found possible to decompose an aqueous solution of carbon dioxide without either an optical sensitiser or a reducing agent, by supplying it with energy in two different ways, viz.: (1) By bombarding it with α- and β-rays from radium emanation and its products, and (2) by exposing it to the light emitted by a quartz mercury-vapour lamp.

The experiment with α- and β- rays was carried out as follows:—About 200 c.c. of distilled water were saturated with carbon dioxide, and into this solution about 0·0001 c.c. of radium emanation was introduced. After four weeks the solution was tested for formaldehyde by Schryver's method.* It contained an appreciable quantity of formaldehyde, a well-marked red colour being observed when the test was applied. The greater part of the aldehyde was in a polymerised form, but no sugar was detected. Another portion of the liquid gave a yellow coloration with a solution of titanium oxide in sulphuric acid, showing the presence of hydrogen peroxide.

The recently published investigations of Kernbaum on the action of β-rays† and of ultra-violet light‡ on water, in which the author stated that hydrogen and hydrogen peroxide were simultaneously produced, suggested an examination of the action of ultra-violet light on solutions of carbon dioxide. In a preliminary experiment, a shallow glass dish containing distilled water was placed immediately beneath, and 2 to 3 cms. from, a quartz mercury-vapour lamp, and carbon dioxide was bubbled through the water while the lamp was in action. After two hours' illumination the water contained hydrogen peroxide, which was identified by the titanium sulphate reaction, as well as a small quantity of formaldehyde. A blank experiment, without carbon dioxide, was then carried out for the same length of time, but in this case formaldehyde was again detected, in addition to hydrogen peroxide. This may have been due to the presence of atmospheric carbon dioxide, but since it was possible that the formaldehyde might have been formed as a decomposition-product of dust particles from the air or the water, the experiment was repeated with greater precautions.

* 'Roy. Soc. Proc.,' B, vol. 82, p. 226.

† 'Comptes Rendus,' 1909, vol. 148, p. 705.

‡ *Ibid.*, 1909, vol. 149, p. 273.

Two transparent quartz tubes, of about 20 c.c. capacity, were filled with the purest "conductivity" water obtainable, and the tubes were inverted in a trough of mercury and placed symmetrically near the mercury-vapour lamp. The water in one of the tubes was as nearly as possible gas-free, and a few cubic centimetres of carbon dioxide were introduced into the other. Both tubes were illuminated for about 12 hours, and the contents of each were then examined for the presence of formaldehyde and hydrogen peroxide. The solution of carbon dioxide was found to contain an easily recognisable quantity of formaldehyde, most of which was in a polymerised form, whereas none, either free or polymerised, could be detected in the water from the other tube. Traces of hydrogen peroxide were present in both. All the reagents used were carefully tested, and negative results were obtained with a solution of carbon dioxide which had not been exposed to ultra-violet light.

It appears from these experiments that ultra-violet light can effect a measurable decomposition of aqueous carbon dioxide without the intervention of an optical or chemical sensitiser, whilst under normal conditions some such agent is required;* moreover, the results furnish very strong support for the belief that both formaldehyde and hydrogen peroxide are formed in a green leaf.

A considerable number of experiments which have been carried out with chlorophyll films point to the same conclusion, and the results of four which may be regarded as typical are tabulated below:—

Description of Experiment.	*Remarks.*
(i) Sealed tube containing chlorophyll painted over a layer of gelatine, made up with catalase solution, on a glass plate. Air and caustic potash present. (ii) The same as (i), but without catalase.	Both these tubes were set up at the same time, at 5 P.M., 22/4/09. At 6 P.M. (ii) showed signs of bleaching. At 12 noon, 23/4/09, (ii) was much bleached, whilst (i) was still quite green and distorted with bubbles.
(iii) The same as (i). (iv) The same as (i), but with a solution of carbon dioxide instead of caustic potash.	Both set up at 11.30 P.M., 24/4/09. At 11 A.M., 25/4/09, (iv) was considerably bleached and developed a very strong coloration after 5 minutes' immersion in Schiff's reagent. (iii) was still quite green, and showed the faintest coloration only after 15 minutes' immersion.

* Berthelot and Gaudechon ('Comptes Rendus,' 1910, vol. 150, p. 1690) obtained formaldehyde by the action of ultra-violet rays on carbon dioxide in the presence of a reducing agent. Such a reducing agent may have been present in our experiments in the form of hydrogen resulting from the decomposition of water (Kernbaum, 'Comptes Rendus,' vol. 149, p. 273, and Tian, *ibid.*, 1911, vol. 152, p. 1012). See also in this connection Stoklosa and Zdobnicjý, 'Anz. kais. Ak. Wiss. Wien,' 1910, No. 19, p. 319.

The above are given as a specimen of a large number of experiments of a similar nature, the results of which leave no doubt in our minds that the bleaching of chlorophyll in sunlight, whether carbon dioxide is present or not, is due to the formation of hydrogen peroxide. The direct oxidation of chlorophyll, in the absence of water, gives rise to a brown and scorched appearance, very different from the true bleaching observed when water is present. As regards the production of formaldehyde, the experiments are equally conclusive in showing that it is only detected by Schiff's reagent when carbon dioxide is present. Great care must be taken to remove any carbon dioxide dissolved in the films used in control experiments.

(b) *In the Plant.*—Generally the same results have been obtained with green tissues, although in this case the observations are not quite so uniformly consistent as in the film experiments, owing to the greater difficulty in controlling experimental conditions. None of these experiments are recorded, since their evidential value is certainly inferior to that of the more easily controlled extra-cellular experiments.

It may be as well to state here that, although no results have been obtained which require any substantial alteration in the theory originally advanced, the following modifications of the conclusions given in Parts I and II have been found necessary:—(1) The statement that the "catalase' enzyme is exclusively localised in chloroplasts and amyloplasts must be abandoned. It appears to have been based on a careless observation, and subsequent experiments have merely indicated a greater concentration of the enzyme in the chloroplasts, for when the green juice obtained by pounding fresh leaves in a mortar is filtered, the green residue containing the chloroplasts decomposes hydrogen peroxide vigorously, whereas the filtered juice is relatively inactive. (2) The bleaching of chlorophyll, whether in or outside of the plant, does not require the presence of carbon dioxide; there is, however, now even more reason to believe that the process is dependent on the formation of hydrogen peroxide.

The whole problem of the production of formaldehyde, both in the plant and in artificial arrangements, can be more satisfactorily dealt with in the way described and experimentally illustrated by Schryver,* and the method may be employed to yield quantitative results. All the experiments recorded in this paper in which Schiff's reagent was used to detect the aldehyde were performed before the work of Schryver was published.

* *Loc. cit.*

2. *The Evolution of Oxygen.*

(a) *In Vitro.*—A method of showing the evolution of oxygen from chlorophyll films in contact with catalase, different from that previously described, has been devised by making use of Beijerinck's luminous bacteria. A pure culture of these bacteria has been observed to glow only in the presence of free oxygen. The experiment, which is described below, can be easily repeated, and involves no troublesome manipulation. The smaller part of a glass Petri dish was divided into two compartments by cementing a narrow strip of cork across the middle. A culture of luminous bacteria in nutrient gelatine was poured into one compartment, and part of the same culture containing some sheep's liver catalase into the other. When the gelatine was set, a film of chlorophyll was painted evenly over both halves, and the lid was put on. The cell was then sealed by pouring melted paraffin wax into the annular space between the rims of the dish and its cover, and gold size was then poured round on top of the wax, by which means the cell was made quite air-tight. It was placed now in a dark room, and both halves were seen to glow with equal brightness, which gradually diminished as the oxygen in the imprisoned air was used up. After two days, no glow could be detected in either half, even after 15 minutes' examination in the dark room. At this stage the cell was taken out and exposed to light for five minutes, and then brought back to the observer in the dark room, when both halves were seen to be feebly glowing, but with unequal brightness. When this glow had again ceased, the experiment was repeated with a different observer, and with the same result. On comparing notes, it was found that each observer had noticed a somewhat brighter glow in the half which contained no catalase. This result was unexpected, but it was subsequently found that the bacteria could be made to glow much more brightly by adding a drop of very dilute hydrogen peroxide than by simply exposing them to atmospheric oxygen, that is to say, they are themselves able to utilise hydrogen peroxide for the light-producing process, and in doing so derive more energy from it than from a direct supply of gaseous oxygen. This experiment therefore not only shows the production of oxygen under the conditions named, but further supports the view that this oxygen is derived from hydrogen peroxide.

(b) *In the Plant.*—The distinction between the behaviour of a plant which had been chloroformed, *i.e.* in which the enzymes had not been destroyed, and that of one in which both protoplasm and enzymes had been killed by immersion in boiling water, was emphasised in Part I, and since more than one writer has failed to confirm the observation therein recorded, that a

small but significant amount of oxygen can still be evolved from a plant which has been chloroformed and subsequently exposed to light in presence of carbon dioxide, the experiment has been repeated in a different form and under more rigorous conditions. As it was essential that no trace of air should remain in the experimental vessel, the latter was exhausted very thoroughly with a small Töpler pump. Fig. 1 shows an arrangement which was found convenient in all such experiments.

The plant was contained in a wide glass tube A, which was then drawn out at the open end and sealed to a piece of narrow tubing thickened to a capillary at *a*. This tube was sealed to a T-piece leading to the pump, and carrying a tube B containing precipitated magnesium carbonate, which was

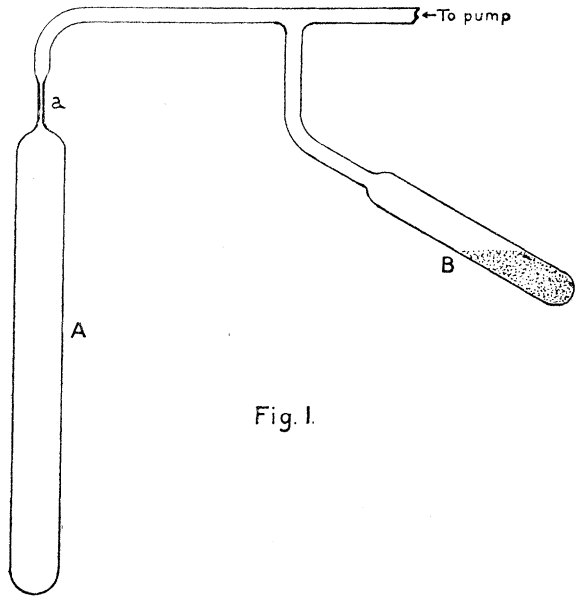

Fig. 1.

employed as a source of carbon dioxide. No stopcocks or rubber connections were used. In the experiment now being considered, some *Elodea canadensis* was chloroformed for two hours, and then placed in A with water and a little thymol (used as an antiseptic). The pump was worked for some time after the last visible traces of gas had been removed, and it is certain that no air remained in the tissues. The magnesium carbonate was now heated, and the system was washed out twice with carbon dioxide. Finally A was filled with carbon dioxide at about 2 cm. pressure, and was sealed off at *a* with a small blowpipe flame. The Elodea was exposed to light for 12 hours, and the tube was then attached to the pump again and thoroughly exhausted, the gas being collected in a tube over mercury. A

small quantity (about 0·2 c.c.) of oxygen was found to be present, and was detected by absorption in alkaline pyrogallol, a result which appears to confirm the statement that the initial stage of photosynthesis can be carried on to a small extent quite independently of living protoplasm. This evolution of oxygen by dead tissues was indeed observed by Molisch* in 1904, in the case of foliage leaves of *Lamium album*, by the luminous bacteria method.

3. *The Absorption of Heat in a Chlorophyll Film, due to Photolysis of Carbon Dioxide.*

It has now been shown that carbon dioxide in the presence of water can be decomposed by ultra-violet light without chlorophyll, and that the same decomposition products can be obtained by the action of ordinary light when chlorophyll is present. Thus there is at least a strong probability that carbon dioxide, and not any constituent of the chlorophyll, is the parent of these decomposition-products. The remaining link in the argument has, however, been supplied by the application of a thermometric test, which is described below. It is clear that, if the assumption is correct, a chlorophyll film in an atmosphere containing moist carbon dioxide should, when illuminated, remain at a lower temperature than a similar film equally illuminated in an atmosphere devoid of that substance, for the production of formaldehyde and hydrogen peroxide from aqueous carbon dioxide involves the absorption of a large amount of heat.

The arrangement devised was a differential one, and served to show the difference between the temperatures of two chlorophyll films set up side by side in two glass tubes which contained the desired gaseous mixture. The apparatus used in the final series of measurements is diagrammatically represented in fig. 2. The chlorophyll films were painted on pieces of thin tinfoil about 1 cm. square (a, a'), which were gummed on to strips of cork (b, b'), which fitted closely in the glass tubes. A single thermo-electric junction was hammered to the back of each piece of tinfoil before the latter was fixed to the cork, and the leads from the junctions, double silk covered and soaked in shellac varnish, passed out at the top of the tubes through a narrow thickened portion of the glass, the passage being sealed by pouring melted paraffin wax into the cups at c, c'. The thermocouples were of copper constantan wire (S.W.G. No. 36), and the weight of the metal substratum of the film, including the hammered-on thermocouple, was about 0·02 grm. per square centimetre. The cork strips served to support the

* 'Bot. Zeit.,' 1904, vol. 62, p. 1.

flimsy metal part, and to diminish to some extent the loss of heat from one surface by radiation and convection. The glass tubes into which the strips fitted were about 15 cm. long and 1·5 cm. in diameter. When an experiment was in progress the tubes were closed at their lower ends by rubber stoppers, on which rested two short tubes (*d*, *d'*), one containing a solution of carbon dioxide in water ("soda-water"), and the other a solution of potassium hydroxide; in this way the films were immersed either in an atmosphere containing moist carbon dioxide or in one devoid of it. The thermo-couples were connected differentially through a reversing key K and a suspended-coil galvanometer G. Any error due to lack of symmetry in the

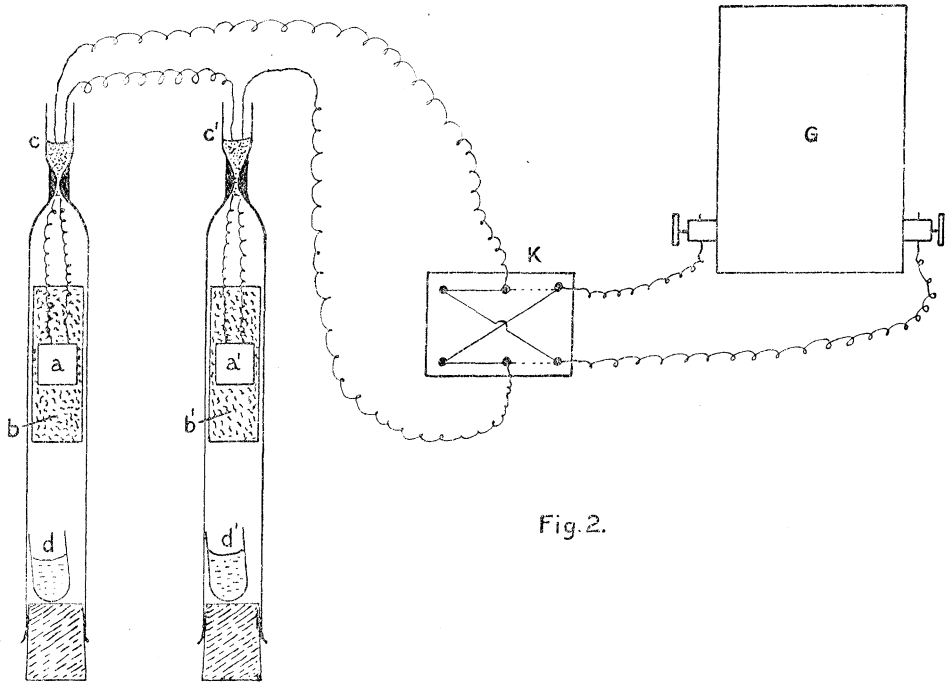

Fig. 2.

thermo-electric behaviour of the system was eliminated by taking always the mean of readings obtained before and after the key was reversed, and differences of temperature due to slight inequalities in the films themselves were allowed for by interchanging the solutions in the tubes *d*, *d'*. The thermocouples were calibrated directly against two mercury thermometers, so as to obtain the number of galvanometer scale divisions per degree difference of temperature.

Some preliminary experiments were carried out in September, 1908, with a slightly different form of apparatus, and one series of observations is given below; the figures do not definitely settle the question at issue,

as no reversal of the films was made, and an important control experiment was omitted; nevertheless they bring out several points of interest not shown in the final series.

One of the films (A) was in CO_2-free air, the other (B) was in a tube connected with a supply of carbon dioxide. With air in both tubes, B was 0°·56 hotter than A when both were exposed to light; in the following table of readings the observed temperature difference is corrected for this want of symmetry :—

Time.	$t_A - t_B$.	Time.	$t_A - t_B$.
CO_2 slowly entering B.		CO_2 in B.	
P.M.	°	P.M.	°
1.30	0·57	3.22	0·43
1.36	1·42	3.23	1·00
1.40	1·70	3.24	1·28
1.45	1·56	3.25	1·42
1.48	0·85	3.26	1·56
(Both tubes were shaded till 2.3)		3.27	1·56
2.3	0·14	3.28	1·42
2.4	0·28	3.30	1·28
2.6	0·43	(Both shaded for 4 minutes)	
2.10	0·57	3.34	0·71
2.14	1·00	3.36	1·28
2.20	1·28	3.37	1·85
2.22	1·28	3.38	1·56
2.24	0·43	3.39	1·28
2.30	0·14	3.42	1·14
2.32	0·00	3.44	1·14
2.34	−0·28	3.46	1·13
2.36	−0·57	3.48	1·00
(At this point it was noticed that film A was almost destroyed. Two new films were therefore prepared.)		3.50	0·85
		3.53	0·28

These figures show remarkable variations of the temperature difference with time. It will be noticed that several minutes are required for the maximum temperature difference to be established, and that this difference does not persist for more than two minutes, but gradually falls off until, if the exposure to light is continued uninterruptedly, the temperatures of the two films become equal, and ultimately the one in carbon dioxide becomes hotter than the control film in air. It was always noticed that the film in CO_2-free air was "scorched" and destroyed sooner than the other, and, regarding each film merely as an absorber of heat, it is obvious that the one in which the chlorophyll is more rapidly destroyed must also be the one in which the amount of heat absorbed in unit time falls off more rapidly. This probably explains the ultimate reversal of the temperature difference, for both films were being gradually destroyed, but by the time the film in carbon dioxide had lost its photolytic efficiency the one in air

had become even more inefficient as an absorber of heat than the first. Again, if the films are shaded for an interval at a time when the temperature difference is diminishing, this difference begins to increase again when the exposure to light is renewed, probably because the carbon dioxide and water undergoing photolysis are used up faster than they can diffuse into the films.

A final series of measurements, in which every precaution was taken to avoid known sources of error, was made in April, 1909, with the apparatus already described. The two films were illuminated through a large ground-glass window and the vessels containing them were carefully protected from draughts. The galvanometer was a dead-beat instrument, and a movement of the spot of light over 25·4 scale-divisions corresponded to a temperature difference of 1°. The error in the readings may be taken as $\pm 0°{\cdot}01$. The results are as follows—

Film A—In tube containing potassium hydroxide solution
Film B— „ carbon dioxide „
Exposed to light at 12.15 P.M.

Time.	$t_A - t_B$.	Time.	$t_A - t_B$.
	°		°
12.16	1·30	2.4	−0·31
12.17	1·74	2.5	−0·47
12.18	1·78	2.6	−0·51
12.19	1·86	2.7	−0·71
12.20	1·81	2.8	−0·79
(At 12.30 the solutions were interchanged, and the films were exposed to light again at 2 P.M.)		(Here the carbon dioxide solution was renewed)	
		2.14	−0·67
2.1	0·20	2.16	−0·91
2.2	0·00	2.18	−1·03
2.3	−0·20		

From the figures given above it is evident that the temperature difference observed depends on the composition of the atmosphere surrounding the films, and that, apart from any want of symmetry in the films themselves, the one in air containing moist carbon dioxide keeps at a lower temperature than the one in air free from that gas. The transitory difference in the wrong direction observed when the films were first exposed to light after interchanging the solutions is doubtless due to a little residual carbon dioxide in film B. A possible source of error, due to a difference in the thermal properties of the gases in the two tubes, was examined by washing the chlorophyll films off their metal supports with benzene, and taking readings when the bare metal was exposed to light, the solutions being

interchanged as in the film experiments. The maximum difference of temperature recorded under these conditions was 0°·06, an amount too small to affect the conclusions.

Conclusion.

The present paper is concerned only with the initial stages of the assimilation process, and therefore no reference has been made to the synthesis of sugars or of starch. The particulars in which the conclusions given in Part I require modification have already been noticed—the exclusive localisation of catalase in the chloroplasts is abandoned, and also the dependence of the *post-mortem* bleaching of chlorophyll on the presence of carbon dioxide.

Finally, there now appears to be ample justification for considering that the primary products of the photolysis of aqueous carbon dioxide are formaldehyde and hydrogen peroxide; that the evolution of oxygen is due to decomposition of the latter substance by catalase; and that up to this point the process is entirely non-vital, and can be reconstructed *in vitro*.

In a paper published in 1907, A. J. Ewart ('Roy. Soc. Proc.,' B, vol. 80, p. 30) has criticised most of the experiments and all the conclusions recorded in Parts I and II. As it is impossible to answer all the objections within the limits of a short paper, replies to a few of the more important are briefly indicated below. (i) The experiments upon which Ewart bases his opinion that formaldehyde was not produced as a decomposition-product of carbon dioxide under the conditions named in Parts I and II are quite valueless for that purpose, because he used the reagent (decolourised rosaniline) in such a way that a colouration would inevitably be produced in the material to be tested. Since the sulphur dioxide (used to keep the rosaniline decolourised) escapes from the solution on warming, or when a large surface (compared with the volume) is left exposed to the air, the method employed and described by Ewart (pp. 30—31) is clearly inadmissible. It may be as well to state here that all the materials—gelatine, petroleum, ether, etc.—used for the experiments described in Part II were tested with the same specimen of Schiff's reagent which was afterwards used to detect formaldehyde, and were only employed if found to be initially free from that substance. In view of the more recent experimental work of Schryver referred to in this paper, it seems unnecessary to discuss the subject at greater length here. (ii) The phenomenon of the bleaching of chlorophyll, and its explanation, have already been dealt with (p. 104). (iii) With regard to the production of hydrogen peroxide, Ewart is mistaken in supposing that we were "unaware that the absence of hydrogen peroxide from living cells has been definitely established": on the contrary, it was expressly stated that an enzyme was present whose function was to decompose that substance as fast as it might be formed. Since chlorophyll is itself attacked (bleached) by hydrogen peroxide, the latter has also escaped detection when the enzyme has been destroyed. (iv) The extra-cellular evolution of oxygen.—The experiment described in Part II has been misrepresented in important particulars by Ewart (p. 34), and in his attempt to repeat it the conditions of the original experiment were not observed. (v) The simultaneous production of formaldehyde and hydrogen peroxide is objected to (p. 35) on the ground that these two substances under certain conditions interact, and form carbon dioxide and hydrogen (or, ultimately, carbon

dioxide and water). The difficulty, however, is imaginary, and the result is possible, because (*a*) the position of equilibrium in the reversible change $CO_2+3H_2O \rightleftarrows HCHO+2H_2O_2$ is displaced towards the right by the addition of light energy, and (*b*) the process is continuous so long as the products on the right-hand side are removed, as in a living plant they are.

H. Euler ('Zeits. für physiol. Chemie,' 1909, vol. 59, p. 122) supports Ewart's criticisms, without, however, giving any particulars (*cf.* foregoing paragraph). He also mentions some experiments with solutions of chlorophyll, quinine sulphate, and fluorescein, which gave negative results. This agrees with our own experience, so far, at least, as chlorophyll solutions are concerned.

Mameli and Pollacci ('Atti dell' Ist. Bot. dell' Univ. di Pavia,' Series II, vol. 13) have published a critical memoir in which, in the first place, they re-affirm the possibility of detecting formaldehyde in the living plant: this appears now to be fully confirmed by Schryver (*loc. cit.*). These authors also failed to observe any evolution of oxygen *in vitro* when they repeated the experiment already referred to, but it is possible that, as they stated that they were unable to prepare a specimen of chlorophyll free from formaldehyde, this substance may have interfered with the action of the catalase in contact with the film of chlorophyll, in which case no oxygen would be produced.

Transmission of Amakebe by means of Rhipicephalus appendiculatus, *the Brown Tick.*

By Dr. A. Theiler, C.M.G., Pretoria.

(Communicated by Colonel Sir D. Bruce, C.B., F.R.S. Received April 20,—Read May 18, 1911.)

That the disease in calves of Uganda called Amakebe is identical with East Coast fever had to be concluded after the presence of the so-called blue bodies of Koch, or plasma bodies, had been demonstrated in the internal organs; these bodies represent certain stages, agametes, agamonts, and gamonts, in the life cycle of *Theileria parva*. Accordingly, it had to be expected that Amakebe could be transmitted by means of such ticks, which act as hosts for this parasite. The most common tick of Uganda is the Brown Tick *Rhipicephalus appendiculatus*, which has been proved in South Africa to be the principal transmitter of East Coast fever.

When in Uganda in 1909 an arrangement was made between Mr. Hutchins, the Government Veterinary Surgeon of Uganda, and myself, to place adult brown ticks, collected as nymphæ from calves suffering from Amakebe, on susceptible calves in my laboratory in Onderstepoort, Pretoria, Transvaal; these ticks were to be collected by Mr. Hutchins as opportunity occurred.