100 mg of glycogen/100 g wet weight are synthesized. Removal of potassium ions from the medium leads to a loss of 11.6 mequiv. K from the tissue, and 173 mg of glycogen are synthesized. An increase of potassium ions in the medium to 17.6 mequiv./l lowers the potassium loss from the tissue, but is without effect on the synthesis of glycogen. The role played by sodium in glycogen synthesis is discussed, together with questions connected with the synthesis of glycogen and the entrance of potassium into the cell.

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THE INHIBITION OF PHOTOSYNTHESIS BY SODIUM FLUORIDE

I. THE SODIUM FLUORIDE-INDUCED CARBON DIOXIDE BURST FROM *CHLORELLA**

NORMAN I. BISHOP AND HANS GAFFRON
Research Institutes (Fels Fund), University of Chicago, Chicago, Ill. (U.S.A.)

Selective inhibition of certain enzymic steps by fluoride-magnesium phosphate complexing would be a rather obvious means to look for possible differences between the well-known metabolic transformations of phosphoglyceric acid (PGA) in glycolytic systems and its photochemical reduction during photosynthesis. As we, and perhaps many others, have experienced, fluorides penetrate very slowly into green algae under the experimental conditions of approximately neutral media. The results indicated a rather complex disturbance of various parts of the general (cell) metabolism. Published experiments[1,2] have shown that photosynthesis is indeed sensitive towards the presence of fluorides in the cell. We found, for instance, after anaerobic fluoride treatment, an inhibition of fermentation and a decreased capacity for photoreduction. At times the inhibition was displaced by a period of photosynthesis in air; at other times such was not the case, *i.e.*, photosynthesis was completely inhibited[3].

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The question of a specific inhibition of some photosynthetic reaction by sodium fluoride has become more interesting for two reasons. First, it has been claimed by KANDLER[1] that PGA is not the substrate for the photochemical reduction step in photosynthesis but that it is formed as a more or less normal derivative from a $C_5 + C_1$ precursor, which would be the true intermediate. Second, WARBURG *et al.*[5,6,7,8,9] have succeeded in obtaining an immediate burst of carbon dioxide upon the addition of sodium fluoride to acid-grown *Chlorella* (at pH 4.0). This work forms part of a series of papers which appeared quite recently and deals with what the authors call the oxygen-capacity and the carbon dioxide-capacity of *Chlorella*. The quantity of carbon dioxide released under the action of fluoride was reported to be related stoichiometrically to the chlorophyll content of the cells in the ratio 1:1.

As a consequence of this, and in continuation of other work on the influence of fluorides* on the mechanism of photosynthesis, we found it necessary to explore this reported phenomenon. Our experimental results show that there is no equivalence between the quantity of carbon dioxide expelled by sodium fluoride and the chlorophyll concentration in our own particular acid-tolerant algal strain. That a burst of carbon dioxide does occur has been verified; but, contrary to WARBURG's findings, the amount of carbon dioxide released can be varied at will according to the conditions chosen. The yield when expressed in equivalence to the chlorophyll present in the algae may vary from values of zero to more than four. A simple experiment proved that in its precursor form, the carbon dioxide released by sodium fluoride is not a substrate for the photochemical reaction. Actually the source appears to be some organic acid whose decarboxylation can be inhibited by cyanide, and it is more closely linked to respiration and fermentation than to photosynthesis. The remaining question is then in which way, if at all, this substance might be connected with the photosynthetic system. An inhibition of photosynthesis as such by sodium fluoride does not answer the question because other photochemical reactions in living cells are also susceptible to such inhibition[10].

What we have observed concerning the effect of sodium fluoride on the rate of photosynthesis proper and on the Hill reaction—which we shall publish in another paper—turned out to be intricate. The effects can vary from a strong inhibition to no inhibition at all, even in cases where a carbon dioxide gush was evident.

MATERIAL AND METHODS

In most experiments, we used the acid-tolerant *Chlorella pyrenoidosa*, strain Marburg St. Several other algae were also studied.

The culture medium had the following composition:

S_1 Medium		
KNO_3	16.2	g
NaCl	9.4	g
NaH_2PO_4	8.8	g
$CaCl_2$	0.36	g
$MgSO_4 \cdot 7H_2O$	4.94	g
EDTA-Na_2	1.0	g
Glass-distilled water	20.0	l

The pH of this solution was adjusted to 4 by adding 0.1 N H_2SO_4. This medium is less concentrated than that employed by WARBURG[6], and also includes the calcium salt in addition to 1 ml/l of a

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* No attempt will be made in this paper to distinguish between the actions of HF, NaF or F^- on the experiments under consideration.

minor element solution having the following composition: Mn as $MnSO_4$, 2 p.p.m., B as H_3BO_3, 1 p.p.m.; Zn as $ZnSO_4$, 0.05 p.p.m.; Cu as $CuSO_4$, 0.01 p.p.m., and Mo as MoO_3, 0.01 p.p.m., and 1 ml of iron solution containing the following: 0.25 g of Fe $(NH_4)_2(SO_4)_2 \cdot 6H_2O$ dissolved in 50 ml distilled water.

After autoclaving, 500 ml of the culture solution were inoculated from agar slants and aerated with 4% carbon dioxide in air. The cultures were grown in large aerating flasks at 25–26°C over white fluorescent lamps at two intensities—300 and 600 foot-candles. As a rule, better cultures (more uniform growth) were obtained at the lower light intensity.

Matured algal cultures (5–7 days old) were harvested by centrifugation at 1000 *g*. Then the algae were resuspended in what will be termed "S_2 medium". This solution was identical to the culture medium, except that 8.56 g/20 l of ammonium chloride and 11.92 g/20 l of potassium chloride were substituted for potassium nitrate and sodium chloride. The pH of this medium was adjusted to 4.0 with 0.1*N* H_2SO_4. Minor elements and iron were not included.

The wet volume of the algae was determined by centrifugation at 1500 *g* for 30 min in cytocrit tubes. Chlorophyll was extracted in boiling methanol. The chlorophyll–methanol solution was mixed with acetone until the final concentration of acetone was 80%. The concentration of chlorophyll was determined by the method of ARNON[11]. Comparison of this method with that of MACKINNEY[12] did not give appreciable variation (± 5%). Also, comparison of the concentration of pure chlorophyll *a* in 20% methanol–80% acetone and in 80% acetone–water gave good agreement.

Gas changes were measured by conventional Warburg techniques. For the experiments reported in this paper, conical vessels fitted with double side arms and centerwell were used. The gas volume of the vessels ranged between 15 and 16 cm^3. In general, the gas phase for all experiments was either pre-purified tank nitrogen or a mixture of 2% carbon dioxide–98% nitrogen. The vessels were agitated at 120 strokes per min with an amplitude of 3 cm.

Photosynthetic measurements were performed by utilizing the reaction between hydrogen and oxygen as catalyzed by palladium[13]. As a check against this method (where 4% carbon dioxide–96% hydrogen was employed), photosynthesis was also measured in 4% carbon dioxide–air. By using the photosynthetic quotient, $[-CO_2/O_2] = 0.96$, practically identical results were obtained by the two methods for determining photosynthetic rates.

Illumination was provided either by a 500 W projection bulb which gave a white-light intensity of 700 foot-candles, or by a bank of four 150 W projector-flood bulbs having a white-light intensity of 1200 foot-candles.

The general reaction system contained between 100 and 400 mm^3 cells (depending on the density of the culture) in 2 ml of S_2 medium at pH 4.3. One-half ml of sodium fluoride (final concentration $= 4 \cdot 10^{-3} M$) was placed in one sidearm and either ½ cm^3 of another addendum was in the other sidearm, or ½ cm^3 of S_2 medium was added to the main compartment of the vessel, to bring the total volume of liquid in the cell to 3 ml. All measurements were performed at 25.3°C. As a general procedure, the vessels were flushed for 10 min with the particular gas desired. Exceptions to this general scheme will be noted.

RESULTS AND DISCUSSION

A. Selection of algae for fluoride studies

It has been the general procedure for researchers in the field of photosynthesis to use as their experimental material the two morphologically and physiologically similar green algae, *Scenedesmus obliquus* and *Chlorella* (either *C. pyrenoidosa* or *C. vulgaris*). As a result, an appreciable proportion of the present-day theories rests upon evidence accumulated from studies on these two organisms. The choice of the experimental material for our work has been dictated by the fact that only *Chlorella pyrenoidosa*, strain Marburg St., and, to a lesser extent, *Chlorella pyrenoidosa*, strain Burk and *Chlorella variegata*, grow well at low pH and these, so far, were the only algae among several studied that gave the sodium fluoride-induced carbon dioxide burst to any satisfactory extent.

Several green algae were examined in this respect, including *Chlorella p.* Emerson, *Scenedesmus obliquus* strain D_3, *Ankistrodesmus braunii*, and *Chlamydomonas moewusii*, strain (—). Neither when grown on media prescribed for each individual alga, nor in acid media where growth was poor, did any of these algae give a fluoride-induced carbon dioxide burst.

All of the above listed algae were also tested after 5–7 days of normal growth by suspension in acid-growing medium used for *C.* Marburg St. (S_1 medium). After several hours (2–24 h) under otherwise normal growing conditions (light + 4% CO_2–air), none of these algae showed any appreciable carbon dioxide burst. Using younger cultures (3–4 days) and treating as above, some experiments gave evidence of a burst. In such cases the burst size was always smaller than that calculated on a chlorophyll–carbon dioxide ratio of 1:1. It was also observed that a decrease in the chlorophyll content occurred when algal samples were resuspended in the acid medium and allowed to photosynthesize. This decrease in chlorophyll content did not affect the burst size. In a similar pattern, the acid-tolerant alga, when transferred from its acid growing solution into less acid media, gradually lost its sensitivity toward sodium fluoride and gave less and less carbon dioxide production when treated with the same concentration of sodium fluoride. Thus, we are not at all sure whether anaerobic carbon dioxide bursts of three to four times the size of a chlorophyll equivalent are the largest ones we may be able to find. Further experimentation with culture conditions may result in still larger ones.

B. The relationship between chlorophyll concentration and the amount of carbon dioxide produced

The amount of carbon dioxide produced by the addition of sodium fluoride to acid-grown cells, under anaerobic conditions, in the experiments of WARBURG *et al.*, is seemingly related to the amount of chlorophyll present in the cells. The stoichiometric ratio they invariably find is 1:1. With *Chlorella* Mbg. treated in the way described above, we have come across this ratio occasionally, but interspersed between numerous other results which deviated appreciably from this value.

As is so well known, EMERSON AND ARNOLD[14] showed that under the most favorable conditions the chlorophyll in the living algae is capable of reducing only a fraction of the equivalent amount of carbon dioxide at any one time. This makes it *a priori* extremely likely that in the type of experiment we are discussing here, the appearance of a 1:1 ratio is purely fortuitous.

In Table I a tabulation of the results of several experiments is given, showing the relationship between the amount of carbon dioxide produced and the quantity of chlorophyll present for each individual experiment. It is quite apparent from the results presented that the amount of carbon dioxide produced varies anywhere from 53% to 493% on a chlorophyll basis.

Because of the question of pH and permeability in relation to burst size, it is important that the amount of gas evolved upon the addition of sodium fluoride can also be varied by other means. Our normal experimental procedure to determine the burst size included ten-minute nitrogen flushes prior to closing the manometers. This period was followed by a thirty-minute measurement of the normal fermentation rate. Then the sodium fluoride was tipped into the algal suspension in the main compartment of the vessel. Thus the algae remained between 30 and 40 min in the dark under anaerobic conditions before the poison was added. If, however, the time between closing the manometers and the tipping of the sodium fluoride was extended, then the burst size did not remain constant but decreased with time. A typical experiment illustrating this is shown in Fig. 1. The conclusion that can be drawn from such an experiment is evident: choosing the right moment for adding the fluoride makes it

TABLE I

YIELDS* OF CARBON DIOXIDE PRODUCED BY THE ADDITION OF SODIUM FLUORIDE TO ACID-GROWN *Chlorella* CELLS

μmoles chlorophyll	*Calculated CO_2 volume (μl)*	*Actual** CO_2 volume (μl)*	*% of calculated*
4.88	119.0	63.0	53
4.6	107.0	110.0	102
4.43	99.2	157.0	158
4.43	99.2	170.0	171
4.1	93.0	134.0	144
3.3	74.0	123.0	166
3.04	68.0	111.0	163
3.03	67.8	139.0	205
2.37	53.0	66.0	124
2.22	49.7	95.0	191
1.34	30.0	148.0	493
1.15	28.0	89.0	317
0.8	17.0	64.0	376
0.58	13.0	32.0	246

* Yields calculated on the assumption of a 1:1 ratio between chlorophyll and carbon dioxide. All experiments included in this table are for algae kept 30 min under nitrogen prior to the addition of sodium fluoride. Wide variability in yields is due to difference in age of cultures. Better yields were obtained with younger cultures (3–4 days). Poorest yield is for 7-day-old culture.

** The carbon dioxide volumes were obtained by subtracting the amount of CO_2 produced via fermentation from the maximum gas volume produced during the burst. This was necessary, for fermentation was not inhibited completely, or in some cases, not at all.

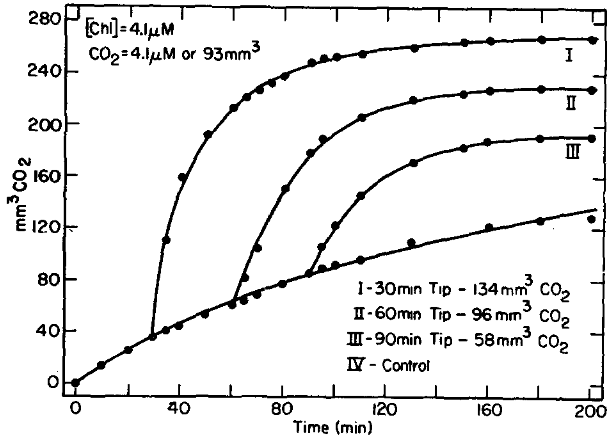

Fig. 1. Curves illustrating the decrease in size of the sodium fluoride-induced carbon dioxide burst as a function of time under anaerobic conditions. The experimental system contained 340 μl of cells (4.1 μmoles of chlorophyll) in the S_2 medium at pH 4.3. Sodium fluoride concentration (final) was $4 \cdot 10^{-3} M$. Temperature 25.3°C. Gas phase = N_2.

possible to obtain—within limits—any desired relationship between carbon dioxide and chlorophyll, or whatever one wishes to relate with the quantity of carbon dioxide expelled.

Closer inspection of Fig. 1 indicates that the size of the burst does not decrease in a 1:1 ratio with the amount of fermentation during a given interval. From the values presented in Table II, the averaged ratio, was 1.6, indicating that for every two molecules of carbon dioxide produced during the gush, approximately 3 molecules of the substance responsible for the gush are used up. In Table II the results of several experiments similar to the one presented in Fig. 1 (and including data from Fig. 1) are tabulated, showing the relationship between time of addition of the poison and the amount of carbon dioxide evolved. Although the ratios given are only approximate, since the actual size of the burst due to the method of determining its magnitude is somewhat arbitrary, it is apparent that the value is closer to 2 than to 1.

TABLE II

TIME OF ANAEROBIOSIS AND YIELD OF CARBON DIOXIDE

	Time of addition*	*Burst (µl)*	*Difference*	*Fermentation (µl 30 min)*	*Ratio***
	30 min	134	38	24	1.57
	60 min	96			
1.					
	60 min	96	38	21	1.7
	90 min	58			
2.					
	30 min	170	33	19	1.7
	60 min	137			
3.					
	0 min	117	54	35	1.5
	30 min	64			
4.					
	0 min	115	33	22	1.5
	30 min	82			
				Average ratio:	1.6 ± 0.1

* Zero time is the closing of the vessels after a 5-min nitrogen flush.

** $\text{Ratio} = \frac{\text{Difference in burst sizes}}{\text{Fermentation}}$

The failure of the burst to remain constant is one of the main points brought out in Fig. 1. If one assumes *a priori* that the CO_2 produced comes from a chlorophyll–carbon dioxide complex, then such data as presented in Table II would prove that such a complex is quite unstable and decomposes rapidly. Such decomposition would be noted as carbon dioxide produced as a result of "fermentation" of algal suspensions, *i.e.*, an increased rate of fermentation until the CO_2–chlorophyll complex is depleted. The addition of sodium fluoride only serves to hasten the decomposition, which we may call a decarboxylation. However, it appears more likely that the source of the carbon dioxide is one (or more) compounds appearing and disappearing during the general metabolism of the cell. All that sodium fluoride does is to induce its immediate decarboxylation. The fairly rapid decrease in the burst size with time is then due to further transformations of this substance during fermentation.

C. *The influence of prior illumination upon the carbon dioxide burst*

According to WARBURG, a stable chlorophyll–carbon dioxide complex is formed with the aid of the much-discussed extra respiration in the light[15]. This, supposedly, is *the* substrate of the photochemical reaction in photosynthesis, and the fluoride experiments are presented as proof of its existence. It is hard to understand why the following simple experiment has not been made to check a hypothesis which so flagrantly contradicts most of the experimental knowledge available at the present time: assuming such a complex does exist, it should be decomposed in the light, but not rebuilt if both carbon dioxide and oxygen are absent. Instead, the amount of carbon dioxide tied up in such a complex should have been transformed into an equivalent amount of oxygen immediately upon illumination. Consequently, the addition of fluoride to algae after illumination under nitrogen should produce little if any carbon dioxide burst. The results of experiments of this kind, made to test these assumptions, are shown in Table III.

TABLE III

INSENSITIVITY OF THE SODIUM FLUORIDE-INDUCED CO_2 BURST TO LIGHT

200 mm^3 algae in ammonium solution; Fluoride = $4 \cdot 10^{-3} M$ (final concentration)

	Size of CO_2 burst (μl) after addition of NaF following pretreatment in						
	a) dark, N_2			*b) light, N_2*			*Increase of light over dark*
Number of experiment	*1D*	*2D*	*3D*	*1L*	*2L*	*3L*	
Time when NaF added							
0 min	92	117	141	114	148	177	
15 min	51			70			24–26%
30 min	—	64	73	—	84	100	
	Loss of burst size by fermentation						
	41	53	68	44	64	77	7–20%

The controls were treated as in Fig. 1. After an initial anaerobic incubation in the dark to insure anaerobic conditions, one of the samples received fluoride immediately, the other two 15 or 30 min later. For the experiments in the light, the samples were first exposed to 700 foot-candles of white light while being flushed with a stream of carbon dioxide-free nitrogen to remove any oxygen released by photosynthesis (which, according to WARBURG *et al.*, could be used to "rebuild" the chlorophyll–carbon dioxide complex). At the end of this illumination period, the vessels were closed and the light turned off. The procedure was then identical for the dark controls.

Besides showing that the substrate for the carbon dioxide gush does not dissociate in a carbon dioxide-free nitrogen atmosphere, these experiments also illustrate that light has no power to decompose, reduce or alter it. Actually, there was always more of it present after preillumination, judging from the slightly larger carbon dioxide bursts, than in the dark controls. But this can easily be understood considering the fact that during the anaerobic incubation in the dark the burst slowly decreases in size. We need only to make the rather obvious assumption that reduction in the light counteracts at least part of this fermentation.

The fermentation of our algae is relatively high (see Fig. 1), thus feeding with glucose did not produce a significant stimulation. But we observed that the presence of glucose in the medium preserved the burst size, just as preillumination did.

D. Fluoride effect under aerobic conditions

Because we found that the action of sodium fluoride in producing a carbon dioxide burst is not directly influenced either by light or carbon dioxide partial pressure, we wondered whether aerobic conditions would greatly alter it. Fig. 2 shows such an experiment. Addition of sodium fluoride to respiring algae produces gushes of the same kind. Sometimes, however, these carbon dioxide gushes are initially small, but respiration in the dark helps to increase the effect. This is quite the opposite of the effect of fermentation.

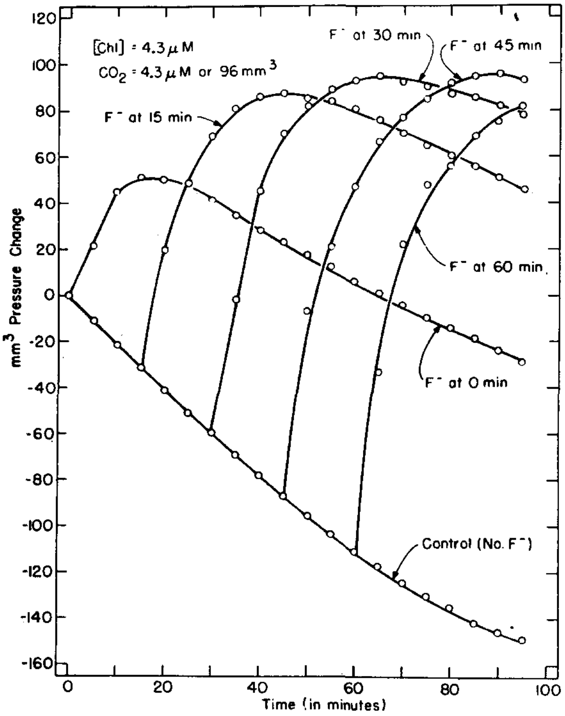

Fig. 2. Curves showing the dependency of burst size upon the length of the aerobic dark period prior to the addition of NaF. The experimental system contained 300 μl of cells/vessel (containing 3.84 μmoles of chlorophyll) in the S_2 medium, pH = 4.3. The sodium fluoride concentration (final) was $4 \cdot 10^{-3} M$. Temperature 25.3°C. Gas phase = air.

E. The effect of sodium fluoride on photosynthesis

After the algae have been treated in a way that gives a good burst of carbon dioxide, photosynthesis was found to be inhibited in some experiments. In others, although the usual burst was observed, no inhibition occurred. Also it was observed at times that sodium fluoride when added to acid-grown algal cells produced neither a carbon dioxide gush nor an inhibition of photosynthesis. And, to complete the possibilities, it was also observed that complete inhibition of photosynthesis could be obtained without the occurrence of a carbon dioxide burst. A more detailed account of such results will be presented in another paper.

The above indicated exceptions to the rule that an accomplished carbon dioxide burst—the sign that sodium fluoride has penetrated into the cell—means automatically a complete inhibition of photosynthesis suffice to show that the metabolic circumstances are much more complex than a simple relationship between carbon dioxide available for a gush and carbon dioxide available for photosynthesis.

The original contention of WILLSTÄTTER AND STOLL (1918) concerning the participation of a chlorophyll–carbon dioxide complex in photosynthesis was based upon the experimental evidence that colloidal chlorophyll suspensions were able to absorb carbon dioxide (0.25 mole CO_2/mole chlorophyll) reversibly. This has not been confirmed in more recent years; RABINOWITCH[16] and SMITH[17] found that chlorophyll and its various derivatives have some reversible affinity for small amounts of carbon dioxide, but also an approximately equal affinity for other gases.

WARBURG's revival of WILLSTÄTTER AND STOLL's hypothesis (for which no further evidence has been presented in the intervening forty years) in the special form of an energy-rich chlorophyll–carbon dioxide complex which is transformed into the end products of photosynthesis by the absorption of one single light quantum, completely contradicts essential features of the photochemical system as it is known at the present time. The experiments described above do not support the notion of a chlorophyll–carbon dioxide complex. Not only was an exact stoichiometry on such a basis seldom realized, but the yield was often several times greater than the amount of chlorophyll present. It is difficult to imagine, then, that any portion of the chlorophyll was photochemically inactive. Furthermore, the manifestation of the sodium fluoride effect depends upon specific growing conditions as well as upon the suspension of the algae in acid media. A particular form of metabolism may be necessary to produce an increased permeability to sodium fluoride. Such a metabolism might result in the accumulation of compounds which, under the influence of fluorides, might be drained away in a spontaneous decarboxylation reaction.

The literature on carboxylation reactions and the enzymic fixation of CO_2 would not be so extensive as it is after only twenty years of research, if these reactions were simple. A reaction between ribulose diphosphate and carbon dioxide resulting in the formation of phosphoglyceric acid (PGA) is generally assumed to be the particular carboxylation which provides the substrate for the photochemical step in photosynthesis. We can expect that this carboxylation, like others, involves more than one reaction on the surface of an enzyme. Therefore, FAGER *et al.*[18, 19], in 1950, when the favorite proposal was to insert a typical $C_3 + C_1$ step into the scheme of photosynthesis pointed out that not only would the regeneration of the carbon dioxide acceptor have to be accomplished in a cycle of transformations among carbohydrates, but that the extractable product of the carboxylation, PGA, probably had a labile precursor. Interest in these matters has suddenly been revived by the observations of GIBBS AND KANDLER[20] on the distribution of tracer carbon along the carbon chain of freshly synthesized hexoses and by KANDLER's discovery[4] of hamamelonic acid as a carboxylation and reduction product in photosynthesis.

Our interest in the action of fluoride on photosynthesis dates back to the question concerning the production of alanine, which is formed as one of the earliest products of photosynthesis. How it arises from PGA as its substrate when the latter is constantly removed by the action of light is of interest to us. Supposedly alanine is always formed in living cells via pyruvate, which in turn is the product of the action of the enzyme enolase upon PGA. Could there be a by-pass from the above mentioned precursor, going directly to pyruvate? Since sodium fluoride (as well as other fluorides) is a specific inhibitor of enolase, the experiments which ought to be done are obvious. But here we run into trouble because normally fluorides do not penetrate readily into the algal cells. We noted a long time ago that photosynthesis became poisoned by fluoride if the cells were left in contact with it for a longer time under anaerobic conditions[3]. But this was not a satisfactory procedure. In experiments of the kind indicated above, one would like to have a certain and immediate inhibitory action.

It is very likely that further experiments with fluoride, which the discovery by WARBURG of the effectiveness of acid treatment made so much more convenient, may give us better insight into the relationship between substrate and intermediate of fermentation and respiration on the one side and of the Benson-Calvin cycle on the other—particularly since KANDLER's discovery has reopened a chapter which for a while seemed nearly finished.

SUMMARY

1. Using the acid-tolerant alga, *Chlorella* Marburg St., it was observed that the sodium fluoride-induced carbon dioxide burst was present. However, a 1:1 ratio between chlorophyll and carbon dioxide evolved during this burst was seldom realized. Yields upwards of 400% (on a chlorophyll basis) were obtained.

2. It was found that the size of the carbon dioxide burst was not stable and decreased if longer periods of anaerobisity were maintained, and that it increased if the thick algal suspensions were allowed to respire. The source of the carbon dioxide seems to be closer to the intermediary metabolism of respiration and fermentation than to photosynthesis.

3. The source of the carbon dioxide gush could not be removed by preillumination of algal suspensions under a nitrogen atmosphere. Such treatment, on the contrary, helped to stabilize the size of the burst, as did the addition of glucose in the dark. Such results are incompatible with the idea of a chlorophyll–carbon dioxide complex as *the* substrate for the photochemical process.

4. The effect of sodium fluoride on photosynthesis of green algae was found to be complex. In some instances complete inhibition occurred, whereas in other experiments no inhibition occurred although a carbon dioxide gush was evident.

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Note added in proof:

In the Oct. 1957 issue of *Z. Naturforsch.*, WARBURG and co-workers published another paper on the fluoride effect confirming our hypothesis that the carbon dioxide gush is indeed a fluoride-induced decarboxylation of an organic acid. They identified the acid as glutamic acid, which is known to occur in algae in relatively large amounts. Nowhere do they discuss or even mention the results of their previous paper, dealing with the discovery of the CO_2 capacity of *Chlorella* and its stoichiometric relationship to chlorophyll. We can assume, therefore, that this controversial chapter is now closed and that the Willstatter-Stoll idea of a chlorophyll–carbon dioxide complex will be shelved permanently.