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THE EFFECT OF CERTAIN BIOLOGICALLY ACTIVE SUBSTANCES UPON PHOTOSYNTHESIS AND DARK CO_2 FIXATION

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THE EFFECT OF CERTAIN BIOLOGICALLY ACTIVE SUBSTANCES UPON PHOTOSYNTHESIS AND DARK CO_2 FIXATION*

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An investigation of the effect of inhibitors, antibiotics and other biologically active substances on photosynthesis and dark CO_2 fixation might be expected to contribute further to our knowledge of these processes and, in addition, to offer a clue to a better understanding of the mode of action of these chemicals during their interference with metabolism. Therefore, such a study was undertaken using tracer techniques which have yielded considerable insight into photosynthesis and dark CO_2 fixation in recent years.[1]

In this first investigation we have explored only a small part of the field. Some of our experiments are of a preliminary character as a result of our desire to gain a broader orientation within a reasonable period of time. However, since in our opinion the results obtained seemed to open important paths for further research, we think it advisable to report the data and give a short discussion of the phenomena at this time.

Experimental methods

Our experiments were performed with 1 day old cultures of *Scenedesmus obliquus* grown in a medium containing 5 ml. 1 M KNO_3, 2 ml. 1 M $MgSO_4$, 0.5 ml. 1 M K_2HPO_4, 0.5 ml. 1 M KH_2PO_4, 0.25 ml. 1 M $Ca(NO_3)_2$, 1 ml. of trace element solution*** per liter. The algae were centrifuged in a refrigerated centrifuge at 1500 r.p.m. for 20 min., resuspended in a small volume of distilled water, centrifuged again and finally suspended in distilled water to make a 1% suspension.

For photosynthesis experiments, 15 ml. of suspension were preilluminated for 10 minutes in a flat circular 20 ml. vessel (diameter 6 cm.) with parallel glass windows (Figure 1). The substance whose influence on photosynthesis was to be tested was added at the beginning of this period to permit penetration into the cells. Air was bubbled through the suspension at a rate of approximately 500 ml. per minute. Reflector spotlights gave a light intensity of about 7000 f.c. on each side of the vessel. To prevent heating of the algal sample, running-water-cooled infra-red filters were placed between the lights and the vessel.

After the preillumination period, a small volume (50 or 100 μl.) of a 0.024 M $NaHCO_3$ solution containing C^{14} was added. The solutions used had an activity of 200 μc. or 400 μc. per ml. The vessel was immediately closed with a ground glass stopper and shaken in the light for 2 minutes. Then the contents of the vessel were dumped rapidly into 60 ml. of boiling absolute ethanol. The mixture was boiled for about 30 seconds in order to make sure that all cells had been killed and then cooled rapidly by placing in running water. 50 or 100 μl. aliquots of the resulting suspension were "plated" on circular aluminum plates in the conventional way[2] and counted. This gave the total amount of activity fixed in the experiment. Subsequently, the mixtures were centrifuged and the clear supernatant liquids decanted. 50 or 100 μl. of these solutions were plated and counted to determine the amount of radioactive carbon present in soluble products. In all of our two-minute fixation experiments this soluble fraction proved to be 60-80 per cent of the total.

In order to determine which compounds had become radioactive, two-dimensional chromatograms were made.[3] For this purpose the solutions were evaporated to a small volume (~1 ml.) _in vacuo_ and 50 or 100 μl. portions then were placed on Whatman No. 1 filter paper sheets (46 x 56 cm.^{2}) that had been prewashed with 1% oxalic acid followed by distilled water. Phenol-water was used as the first solvent and a butanol-water-propionic acid mixture as the second. Radioautographs were made using Kodak "No-Screen" X-ray film. The various radioactive spots were counted directly on the paper.[4,5] When the identity of the compound giving a radioactive spot was doubtful, it was checked by co-chromatography with carriers and chemical tests after elution from the paper. Finally, a survey was made of the distribution of the activity among the various compounds. This distribution was expressed as percentage of the total amount of radioactivity in the soluble fraction and as the amount of radioactivity fixed per ml. of packed cells.

The dark fixation experiments were performed using comparable techniques. The compound to be tested was added to 10 ml. of a 2% cell suspension. The systems were then kept in 50 ml. erlenmeyer flasks (stoppered) at room temperature in the dark for 150 minutes with occasional shaking. After that time, 100 μl. portions of radioactive $NaHCO_3$ solutions (200 or 400 μc./ml.) were added and the systems left in the dark with occasional shaking for another 40 minutes. After this period, the algae were killed and the resulting mixtures analyzed in the same way as described for the photosynthetic experiments.

Results

A. Dark fixation experiments

Typical examples of the results are compiled in Table I. Each group of Table I represents experiments performed at different times. However, the conditions in all experiments were as nearly identical as possible. Controls are reported for each set of experiments. CO_2 dark fixation was studied under the influence of aminopterin (four other anit-folic substances gave essentially the same result), aureomycin, terramycin, penicillin, pyrithiamin, dinitrophenol, benzothiazol-2-sulfonamide, neomycin and chloromycetin.

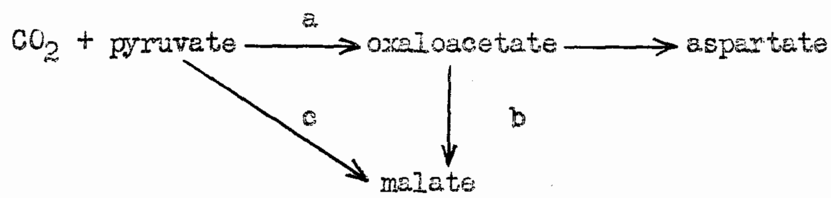
With the antifolics, no influence on dark fixation could be detected, either in the rate of CO_2 uptake or in the nature or the amount of the radioactive compounds formed. Of course, one of the difficulties with all experiments of this type is that it is hard to decide to what extent the added compound penetrated the cell, or whether it has reached the site of possible action.

Dinitrophenol in a concentration of about 10^{-3} M completely inhibited CO_2 uptake. It is even conceivable that it may have killed the algae during the experiment. We have no data on this possibility.

There appears a variability in the effect of those chemicals which have been tested on more than one batch of algae, i.e. penicillin and aureomycin in groups A and B. It seems most likely that this difference is to be attributed to the different physiological states of the algae used in the two sets of experiments. The evidence that such a difference exists lies in the fact that the total fixation by the control of group B is less than half that by group A, and the distribution of radioactivity among the soluble compounds is quite different. There is, however, a certain amount of consistency within each group. When there appears a relatively large ($>\sim 20\%$) departure from the control in the distribution corresponding to some added compound, it seems most likely that this difference is attributable to the effect of that compound under these particular physiological conditions.

The most striking effect on the dark fixation distribution appears in the experiment of group A in which the labeling of malic acid is sharply inhibited by penicillin (somewhat less so by pyrithiamin) while the total fixation is changed very little (Figure 2). The experiments of group B show a similar effect (also for neomycin) but to a much smaller degree.

Enzymatic studies have demonstrated two possible methods of malic acid formation[6,7].



Therefore, the disappearance of malic acid after treatment with penicillin might be explained by a blocking action at either b or c. One might expect that under certain conditions this would lead to increased incorporation into aspartic as appears to be the case in the experiments of group B with penicillin and neomycin.

The results with aureomycin were somewhat varying. With concentrations of 10^{-4} molar or less, we found no appreciable influence on CO_2 uptake although there was slight indication of a slowing down. With concentrations of 10^{-3} M or 2×10^{-3} M retardation of the CO_2 uptake was observed. However, the degree of retardation varied in our experiments, the inhibition in group A being 100%, in group B only 25%. The difficulty with relatively high concentrations of aureomycin hydrochloride is that the algal suspension becomes more acidic. Cell suspensions that had been in contact with aureomycin for several hours during dark fixation experiments carried on photosynthesis at a normal rate after the contact period. Therefore, the cells were not killed during this three hours exposure to aureomycin.

B. Photosynthesis experiments

The results of photosynthesis experiments are presented in Tables II and III. Dinitrophenol in the concentration tested (3×10^{-4} M) stopped photosynthesis as it stopped dark fixation. The antifolics, showing no influence on dark fixation, did not alter photosynthesis. Also folic acid proved to be without measurable effect in a concentration of 2×10^{-4} M. Penicillin and chloromycetin had no influence on photosynthesis with respect to either the rate of uptake or distribution.

We concentrated our research on aureomycin hydrochloride and terramycin hydrochloride. As is shown in Table II, these antibiotics accelerate the rate of photosynthesis under the conditions of our experiments about 2 times when present in a concentration of 5×10^{-5} M (No. 16) and 6 times (No. 17) when present in concentrations of 1.5×10^{-4} M. Higher concentrations (Nos. 18 and 19) gave no greater increase in the amount of CO_2 fixation. It appears as if, at these concentrations, apparent photosynthesis has reached a maximum velocity limited by a factor (light intensity?) that is almost independent of further changes in the reaction medium.

When more stabilized media and better-known conditions were obtained by working in phosphate or ammonium chloride/ammonia buffers no further increase caused by the additions of aureomycin could be demonstrated. In dilute buffers of this type, photosynthesis had already reached a ceiling value. Ammonium chloride in low concentration had a measurable, though somewhat smaller, effect than hydrochloric acid. Most probably it is due in large part to a pH shift and increased buffer capacity.

Hydrochloric acid causes an increase in CO_2 uptake. Experiment 8 demonstrates that the chloride ion does not have a measurable influence at a concentration of 10^{-3} M. Neither does the increase in ionic strength by salt additions. Experiments 14 and 15 in phosphate buffers of pH 7 and 5 respectively suggest that pH in our experiments has an influence on CO_2 uptake.[8] Phosphate has a remarkable influence in rather low concentrations (exp. 12 and 13). Here an increase due to buffer capacity is not unexpected but the effect is somewhat large in order to be wholly accounted for in this way.**** Moreover, a specific effect of phosphate seems not to be improbable since the early carbon compounds of photosynthesis are phosphorylated compounds.

This effect of hydrogen ion concentration probably is due to its influence on the rate of penetration of carbon into the cell rather than to a direct influence on internal metabolism. Part of the effect of the hydrochloric acid salts of both antibiotics can be attributed to a pH shift as aureomycin and terramycin are extremely weak bases. Experiment 9 as compared with 7 suggests that there remains a considerable effect of the antibiotics when this pH shift is accounted for. Experiments 22 and 23, where an equivalent amount of sodium hydroxide was added at the same time as the aureomycin or terramycin hydrochloride, seem to support this view. However, even if, as in these last experiments, there is no shift in the pH at the start of the experiment, we still have no guarantee that the antibiotics will not influence the external CO_2 pressure. By potentiometric titration we found that besides the weakly basic group ($pK_A \approx 3.5$) there is present a group with a pK_A of 7-8 and one with a pK_A around 9 in aureomycin and terramycin. It is the second group (pK_A 7-8) that conceivably could be of influence by its buffering capacity when the radioactive bicarbonate solution is added to the cell suspensions. During the relatively short term photosynthetic experiments, the system is not in equilibrium, nor has it reached a steady state. Determinations of only the total amount of fixation of radioactive CO_2 as influenced by aureomycin and terramycin did not furnish unequivocal evidence for any influence of these antibiotics on photosynthesis other than by their effect on the external medium or the cell membrane. Direct evidence on changes in the internal metabolism of the algae became available after studying the chromatograms.

Detailed information could be expected from the chromatographic analysis of the various radioactive products formed during the photosynthesis. Group A of Table III gives some results of a series of experiments performed on the same day with the same Scenedesmus suspension under circumstances that were as constant as possible regarding light intensity, temperature, technique of analysis, etc. Compare also the reproductions in Figure 3. One of the most striking features is that in all treatments the activity in most of the well-known intermediates (phosphoglyceric acid, carbohydrate diphosphates and monophosphates, nucleotides, serine, glycine, aspartic acid) is increased in the same ratio as the total uptake. The chromatogram from the products of photosynthesis with phosphate present shows an abnormal increase in the amount of alanine formed. This is comparable to the results obtained from cells that have suffered CO_2 starvation before addition of bicarbonate. The picture obtained with aureomycin is rather normal, except for the high activity in sucrose which usually is obtained only in long-term photosynthesis experiments.

From measurements of the amount of total activity fixed, it is evident that part of the effect of aureomycin consists in the elimination of a limiting process. One possible way to account for the general uniformity of the increase in the various compounds is to assume an influence on the rate of penetration into the cells. In addition to this rather general effect, the chromatographic results indicate effects on internal processes during photosynthesis. This could be an acceleration of a process resulting in sucrose formation, inhibition of sucrose utilization or the blocking of another path of carbon.

Group B of Table III presents results from another series of experiments. In this series terramycin HCl and aureomycin HCl were neutralized to pH 7 with NaOH before addition to the cell suspensions. The total amount of CO_2 fixed was lower than in experiments using the corresponding hydrochlorides but higher than in the control. The amount of radioactivity in sucrose was also less than in the hydrochloride treatments but greater than in the control.

The addition of ammonium chloride as well as aureomycin to the cells changed the pattern of radioactive carbon distribution considerably. The radioactivity in aspartic acid, glutamic acid and glutamine increased markedly and the phosphates in general showed a decrease. This is another example of the change of metabolic end products produced by the addition of externally added substances.

C. Growth

The question of whether the accelerating influence of aureomycin extends to the overall rate of growth and cell division was investigated by an experiment in which test-tube cultures of Scenedesmus were grown in culture medium containing 10^{-3} *M* aureomycin hydrochloride in one set and 10^{-3} *M* ammonium chloride in the controls. The pH of the latter was adjusted to that of the aureomycin cultures (pH 5) with hydrochloric acid. Figure 4 shows the results of cell counts made at intervals on both sets of cultures. It is clear that cell division did not occur in aureomycin cultures. No obviously disproportionate increase in size of the aureomycin treated cells occurred. In a separate experiment a crystal of aureomycin hydrochloride was placed in the center of an agar pour-plate of Scenedesmus and the algae allowed to grow in the light. The result was an inhibition zone (~3 cm. diameter) surrounded by a narrow circle in which the algae grew to a greater depth in the agar than they did in the zone still further removed in which the antibiotic presumably had no effect.

Discussion

It is almost superfluous to stress that with the extreme complexity of the interplay of reactions in photosynthesis it is nearly impossible to draw definite conclusions about the kinetics responsible for a certain distribution pattern of activity obtained under a single set of conditions. However, speculations as to probable explanations of the results already obtained will suggest new lines for investigation. For example, consideration of the remarkable increase of sucrose formation by aureomycin and terramycin has led us to the conjecture that antibiotics could in some organisms have a direct acceleration effect on certain steps of metabolism.

One might suggest the possibility that an antibiotic functions positively in a metabolic process (as coenzyme) in the organism where it is produced, while in another organism with a comparable but slightly different enzymatic construction it acts as an inhibitor. If this suggestion is true then it follows that the susceptible organism (to a particular antibiotic) might itself contain a substance having antibiotic properties against the producer of the initial antibiotic. The idea of a positive metabolic function for the substances generally called antibiotics would have considerable significance to genetics and theories of evolution.

Summary

The influence of certain antibiotics and other biochemically active substances on CO_2 fixation in the dark and in the light was studied by C^{14} tracer techniques. Dinitrophenol stopped both processes, whereas anti-folics did not show any influence on the rate or the pattern in CO_2 fixation by *Scenedesmus obliquus* under the conditions of our experiments. Chloromycetin did not alter the velocity of CO_2 uptake in the dark or in the light.

Penicillin did not alter the total uptake of CO_2 but apparently altered the metabolic pattern in dark fixation, e.g. the decrease in the amount of radioactive malic acid formed. In control experiments it was confirmed that malic acid becomes strongly labeled during dark CO_2 fixation. Pyrithiamine had no significant influence on total dark fixation, although it lowered the activity of the malic acid spot.

Aureomycin hydrochloride had an inhibiting effect on dark fixation in concentrations of about 10^{-3} M. It accelerated photosynthesis about two times in 5 x 10^{-5} M concentration and six times in 1.5 x 10^{-4} M and higher concentrations. Terramycin hydrochloride showed a similar behaviour. Probably part of this effect is due to shifts in pH and another part to increased buffering capacity. While it was possible to eliminate the direct pH effect, it is difficult to estimate the influence of the increased buffer capacity. Analysis of the chromatograms of the products formed revealed that the activity in most of the products found in short period photosynthesis was increased proportionally. However, addition of aureomycin produced an abnormally high increase in sucrose and phosphate increased the amount of alanine produced. A short discussion of these phenomena has been given.

References

(1) A. A. Benson and M. Calvin, Ann. Rev. of Plant Physiol., 1, 25 (1950).

(2) M. Calvin, C. Heidelberger, J. Reid, B. Tolbert and P. Yankwich, "Isotopic Carbon," John Wiley and Sons, Inc., New York, N.Y. (1948).

(3) A. A. Benson, *et al.*, J. Am. Chem. Soc., 72, 1710 (1950).

(4) V. H. Lynch and M. Calvin, J. Bact., 63, 525 (1952).

(5) A. A. Benson, S. Kawaguchi, P. M. Hayes, and M. Calvin, J. Am. Chem. Soc., 74, 4477 (1952).

(6) A. G. Wood, Advances in Enzymology, 12 (1951).

(7) S. Ochoa, Physiol. Rev., 31, 56 (1951).

(8) C. Ouellet and A. A. Benson, J. Exper. Bot., 3, 237 (1952).

Footnotes

(*) The work described in this paper was sponsored by the U.S. Atomic Energy Commission.

(**) Prof. of Chemistry, Organic Chemical Laboratory, Royal University, Leiden, Holland.

(***) Composition of trace element solution: 2.8×10^{-5} M H_3BO_3, 4.5×10^{-6} M $MnSO_4 \cdot 4 H_2O$, 3.7×10^{-7} M $ZnCl_2$, 1.6×10^{-7} M $CuSO_4 \cdot 5 H_2O$, 5.6×10^{-8} M $H_2MoO_4 \cdot H_2O$, 1.8×10^{-2} M $FeSO_4 \cdot 7 H_2O$, and 1.3×10^{-2} M tartaric acid.

(****) More extensive investigations in this laboratory by Dr. Dan MacDougall seem to indicate that a very large part of the effect of aureomycin hydrochloride is due to buffering capacity.

Table I

CARBON DIOXIDE DARK FIXATION WITH SCENEDESMUS

10 ml. 2% Scenedesmus (200 λ cells), 150 min. preperiod in the dark, 40 min. dark fixation after addition of 100 λ $NaHCO_3$ (0.024 molar, 200 or 400 μc/ml.), hot alcohol killing, final volume 50 ml.

Group A[1]					
Substance Studied	Control[2]	Pyrithiamin	Penicillin G	Dinitro-phenol	Aureo-mycin HCl
Concentration	--	10^{-3}M	$2x10^{-3}$M	$1.5x10^{-3}$M	$2x10^{-3}$M
Total counts/min. per ml. cells per 40 min.	$1.15x10^5$	$1.05x10^5$	$1.41x10^5$	0	0
Soluble activity/ total activity,%	81.0	73.0	71.0	--	--
% of control fixed	100	92	123	0	0
Vol. CO_2/ml. cells uptake x 10^{-3}	0.98	0.89	1.20	0	0
Distribution of Activity					
Malic acid	8.9	3.5	0	--	--
Aspartic acid	47.5	39.0	52.0	--	--
Glutamic acid	41.0	56.0	45.0	--	--
Citric acid	0.3	0.0	0.9	--	--
Succinic acid	2.3	1.5	2.5	--	--
Fumaric acid	--	--	--	--	--
Sucrose	--	--	--	--	--
Alanine	--	--	--	--	--

(1) Each of these groups constitutes a separate set of experiments done with a different batch of algae.
(2) 200 μc/ml. $NaHCO_3$ used.

Table I (cont'd)

Group B[1]							
Substance studied	Control[3]	Aureomycin HCl	Terramycin HCl	Chloromycetin	Penicillin G	Neomycin sulfate	Benzothiazole 2 sulfonamide
Concentration	--	1.1×10^{-3} M	10^{-3} M	1.6×10^{-3} M	1.8×10^{-3} M	500 mg./liter	4.5×10^{-3} M
Total counts/min. per ml. cells per 40 min.	0.93×10^5	0.70×10^5	0.70×10^5	0.87×10^5	1.01×10^5	1.33×10^5	0.65×10^5
Soluble activity/total activity, %	97.0	--	92.0	93.0	89.0	91.0	95.0
% of control fixed	100	75	75	94	109	143	70
Vol. CO_2/ml. cells uptake $\times 10^{-3}$	0.40	0.30	0.30	0.37	0.43	0.56	0.28
Distribution of Activity							
Malic acid	36.5	--	27.5	39.0	29.6	18.1	33.2
Aspartic acid	7.6	--	7.2	6.8	12.4	13.9	4.7
Glutamic acid	21.3	--	36.6	20.2	24.5	29.6	24.6
Citric acid	4.2	--	2.3	2.6	3.8	1.4	7.5
Succinic acid	18.6	--	18.2	20.0	18.1	24.9	16.1
Fumaric acid	2.2	--	1.7	2.6	2.7	2.9	5.5
Sucrose	--	--	--	--	--	--	--
Alanine	9.8	--	6.4	8.7	9.0	9.4	8.3

(3) 400 μc/ml. $NaHCO_3$ used.

Table I (cont'd)

Group C[1]		
Substance Studied	Control[2]	Aminopterin
Concentration	--	2.5×10^{-4} M
Total counts/min. per ml. cells per 40 min.	5.6×10^{5}	5.2×10^{5}
Soluble activity/ total activity, %	61.0	54.0
% of control fixed	100	93
Vol. CO_2/ml. cells uptake x 10^{-3}	4.9	4.4
Distribution of Activity		
Malic acid	10.0	10.0
Aspartic acid	28.0	38.0
Glutamic acid	32.0	32.0
Citric acid	6.0	6.0
Succinic acid	0.5	0.7
Fumaric acid	--	--
Sucrose	11.0	6.5
Alanine	2.0	1.6

Table II

Effect of various chemicals on two minute photosynthesis with Scenedesmus. 1% suspension; 10 minute preperiod with the substance added. Air bubbling through.

Substance Studied	Conc. x 10^3	ml. CO_2/ ml. cells	% of Control	Soluble/ total	pH at the start of the experiment
1. Control			100		6.3 - 7.3
2. Dinitrophenol	0.3		0		
3. Penicillin	0.6		130		
4. Chloromycetin	0.4		90		
5. Aminopterin	0.2		100		
6. Folic acid	0.2		100		
7. Aureomycin HCl	0.15		600		6.2
8. Potassium chloride	1.0		120		7.0
9. Hydrochloric acid	0.15		300		6.2
10. Ammonium chloride	0.15		200		6.2
11. NH_4Cl/NH_4OH buffer	60.0		600		7.0
12. Phosphate	0.15		200		7.1
13. Phosphate	1.5		600		6.9
14. Phosphate	30.0		300		7.0
15. Phosphate	30.0		600		5.0
16. Aureomycin HCl	0.05		200		~6.5
17. Aureomycin HCl	0.15		600		6.2
18. Aureomycin HCl	0.5		600		~5.0
19. Aureomycin HCl	1.5		600		~3.5
20. Phosphate and aureomycin	1.5 0.15		600		6.9
21. NH_4Cl and aureomycin	60.0 0.15		600		7.0
22. Aureomycin	0.15		200		7.0
23. Terramycin	0.3		500		7.0

24. Terramycin HCl gave approximately the same results as aureomycin HCl

Table III - Group A

2 Minutes Photosynthesis by Scenedesmus with Various Compounds Added

	% of Activity on Paper			
	Control	Hydrochloric Acid Table II #9	Aureo-mycin-HCl Table II #17	Phosphate Table II #12
Origin	2.0%	2.1%	1.9%	1.2%
Nucleotides	5.8	6.2	7.2	8.7
Diphosphates	22.0	24.5	19.3	17.1
Monophosphates right	19.0	19.0	20.0	17.6
Monophosphates left	10.7	9.4	10.1	11.6
Phosphoglyceric acid and Phosphoglycolic acid	4.7	4.3	4.7	5.2
Aspartic acid	4.0	3.7	2.3	6.0
Serine and Glycine	18.0	13.6	8.5	10.6
Sucrose	4.5	6.9	20.7	5.1
Alanine	2.6	4.7	4.2	11.3
Malic Acid	1.9	1.6	1.1	2.2
% activity soluble in hot 80% alcohol	75	80	72	65
	Counts/minute/λ cells			
Origin	26	67	151	86
Nucleotides	76	198	565	608
Diphosphates	285	786	1514	1230
Monophosphates right	246	610	1566	1267
Monophosphates left	138	302	787	836
Phosphoglyceric acid and phosphoglycolic acid	64	136	364	363
Aspartic acid	52	119	180	1196
Serine and Glycine	233	436	657	
Sucrose	58	221	1513	356
Alanine	34	151	329	315
Malic Acid	25	53	90	161

Table III - Group B

	% of Activity on Paper			
	Control	Terramycin Table II #23	Aureomycin Table II #22	Aureomycin + NH_4Cl Table II #21
Nucleotides	9.6%	9.0%	11.2%	4.9%
Diphosphates	6.2	12.6	7.2	9.8
Monophosphates right	7.1	9.7	11.4	4.2
Monophosphates left	7.6	6.5	6.1	5.9
Phosphoglyceric acid	18.5	14.7	19.6	3.4
Phosphoglycolic acid	2.2	1.6	1.5	--
Phosphopyruvic acid	2.2	1.9	2.1	--
Aspartic acid	10.6	8.7	8.3	20.8
Serine	4.4	7.2	7.4	5.9
Glycine	5.1	2.4	2.4	0.9
Sucrose	1.0	1.6	2.3	--
Alanine	4.8	5.0	4.5	4.1
Malic acid	19.5	17.7	14.9	16.8
Glutamic acid	0.8	1.0	0.9	5.0
Citric acid	0.2	0.6	0.4	0.5
Glutamine				4.1
Unidentified				12.7

Table III - Group B (Cont'd)

	Counts/minute/ cells			
	Control	Terramycin Table II #23	Aureomycin Table II #22	Aureomycin + NH_4Cl Table II #21
Nucleotides	159	487	528	308
Diphosphates	103	680	340	645
Monophosphates right	117	524	538	276
left	126	350	289	388
Phosphoglyceric acid	306	790	927	222
Phosphoglycolic acid	36	84	72	----
Phosphopyruvic acid	37	102	97	----
Aspartic acid	175	470	391	1360
Serine	74	386	351	388
Glycine	84	130	112	61
Sucrose	17	84	110	----
Alanine	80	268	212	269
Malic acid	322	956	705	1100
Glutamic acid	14	52	41	325
Citric acid	4	33	20	97
Glutamine	----	----	----	268
Unidentified	----	----	----	834

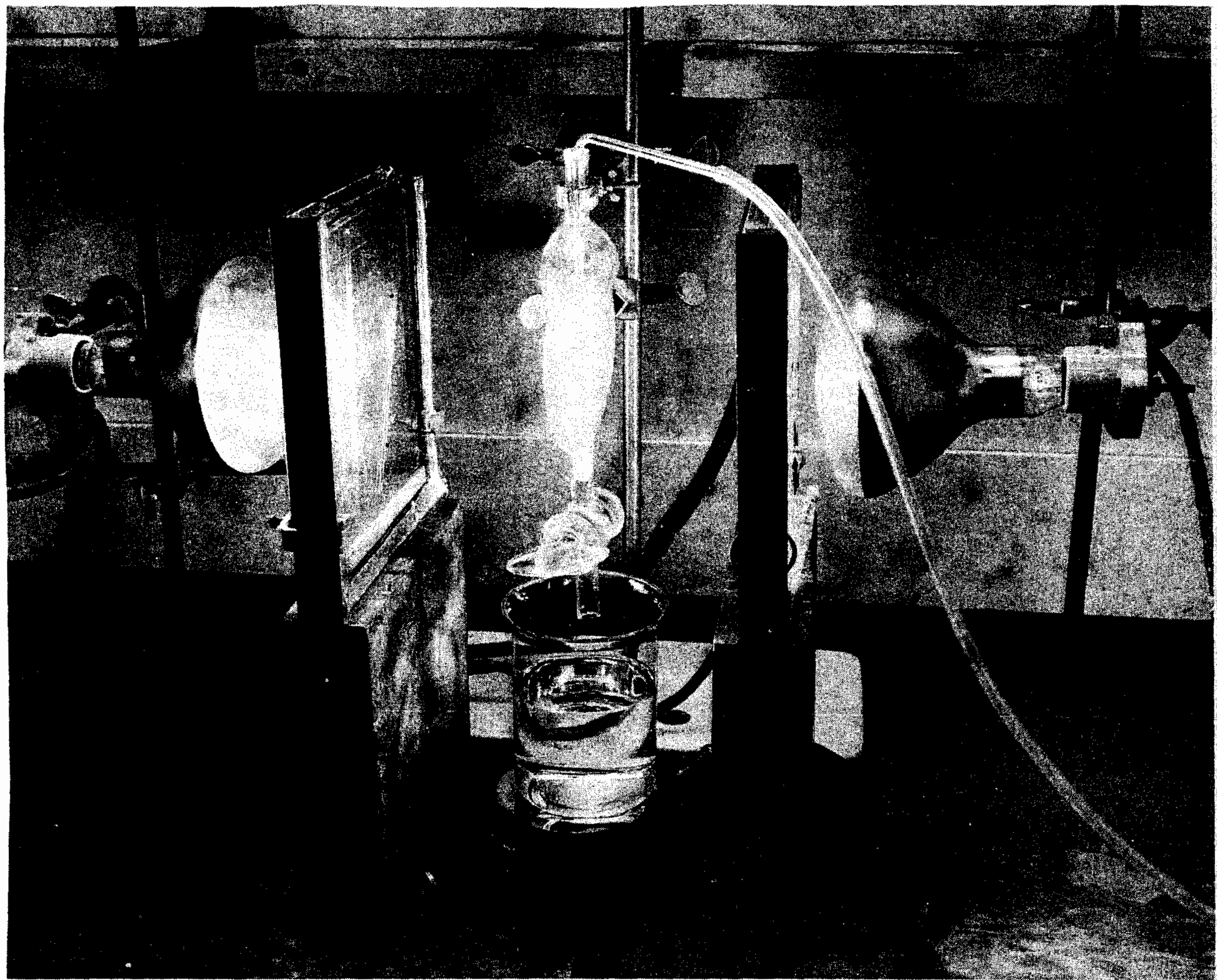
Captions to Figures

Figure 1 - Illumination vessel for photosynthesis experiments

Figure 2 -

Figure 3*- (*) Because of the initial acidity of the paper, aspartic, malic and glycollic acids ran farther than usual in the phenol (horizontal) direction and alanine did not run as far.

Figure 4 -



ZN-481

Fig. 1

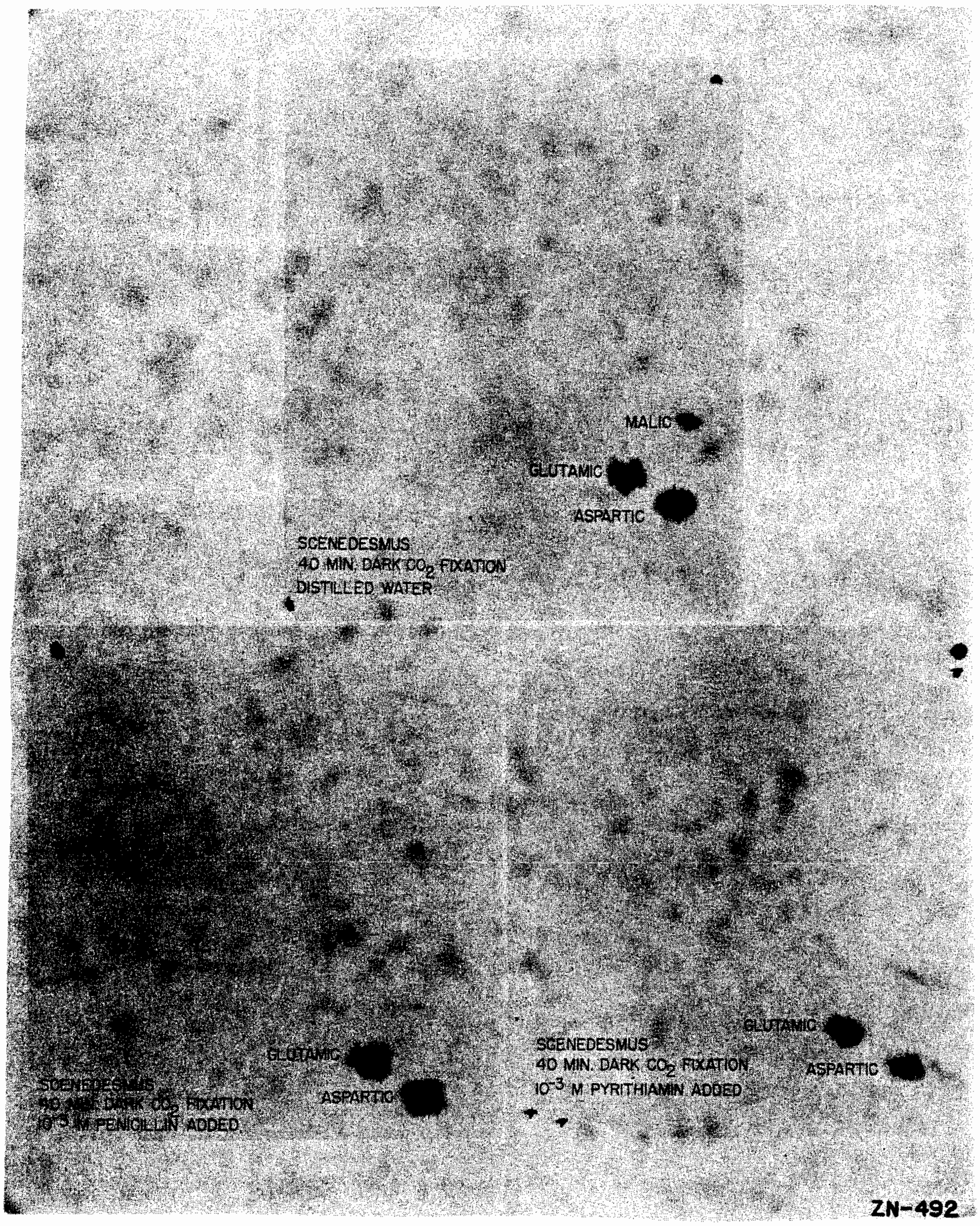
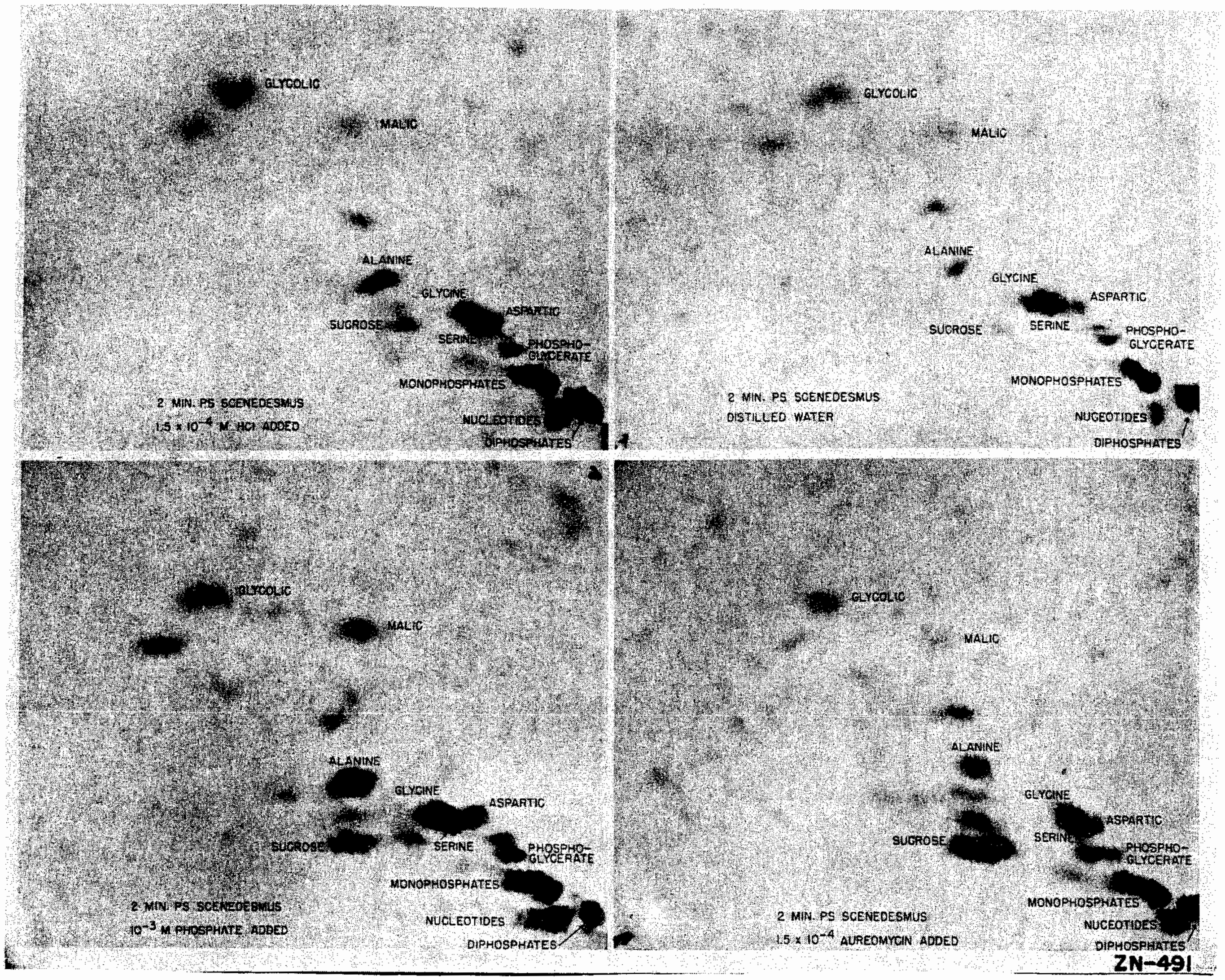
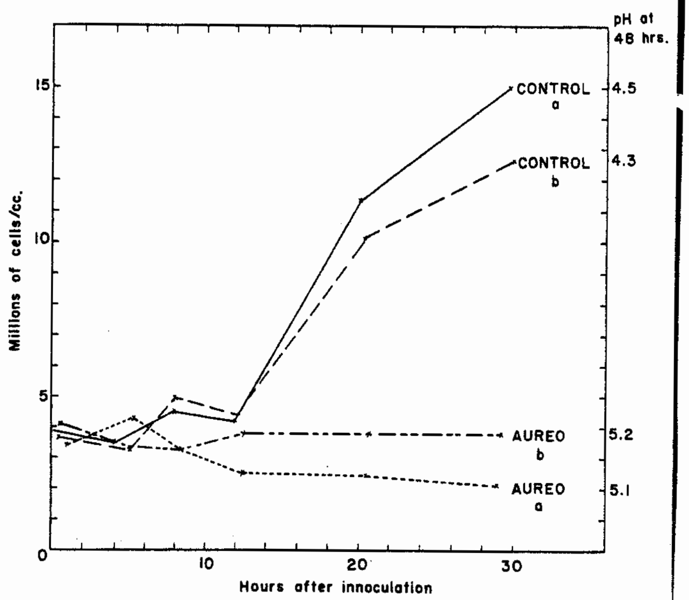

MALIC
GLUTAMIC
ASPARTIC
SCENEDESMUS
40 MIN. DARK CO2 FIXATION
DISTILLED WATER
GLUTAMIC
ASPARTIC
SCENEDESMUS
40 MIN. DARK CO2 FIXATION
10-3 M PENICILLIN ADDED
GLUTAMIC
ASPARTIC
SCENEDESMUS
40 MIN. DARK CO2 FIXATION
10-3 M PYRITHIAMIN ADDED
ZN-492

Fig. 2



GLYCOLIC
MALIC
ALANINE
GLYCINE
ASPARTIC
SUCROSE
SERINE
PHOSPHO-GLYCERATE
MONOPHOSPHATES
NUCLEOTIDES
DIPHOSPHATES
2 MIN. PS SCENEDESMUS
1.5×10^{-4} M HCl ADDED
GLYCOLIC
MALIC
ALANINE
GLYCINE
ASPARTIC
SUCROSE
SERINE
PHOSPHO-GLYCERATE
MONOPHOSPHATES
NUCEOTIDES
DIPHOSPHATES
2 MIN. PS SCENEDESMUS
DISTILLED WATER
GLYCOLIC
MALIC
ALANINE
GLYCINE
ASPARTIC
SUCROSE
SERINE
PHOSPHO-GLYCERATE
MONOPHOSPHATES
NUCLEOTIDES
DIPHOSPHATES
2 MIN. PS SCENEDESMUS
10^{-3} M PHOSPHATE ADDED
GLYCOLIC
MALIC
ALANINE
GLYCINE
ASPARTIC
SUCROSE
SERINE
PHOSPHO-GLYCERATE
MONOPHOSPHATES
NUCEOTIDES
DIPHOSPHATES
2 MIN. PS SCENEDESMUS
1.5×10^{-4} AUREOMYCIN ADDED
ZN-491

Fig. 3



EFFECT OF AUREOMYCIN ON CELL DIVISION OF SCENEDESMUS

Fig. 4