TECHNICAL PAPERS

The Path of Carbon in Photosynthesis: II. Amino Acids[1]

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The amino acid constituents of the green algae *Chlorella pyrenoidosa* and *Scenedesmus* D-3 have been examined after exposure to $C^{14}O_2$, using the method of paper chromatography. Not only have the free amino acids been identified, but the radioactive members of the group have been ascertained.

The methods used in these experiments, which have been previously described (*2, 3*), involve the preparation of filter paper chromatograms of whole-cell extracts (80% ethanol) or of amino acid mixtures obtained by absorption on cation exchange resins from the plant extracts (*1*). The paper chromatograms of the radioactive amino acids were either scanned with a Geiger counter or radioautographed.

In *Scenedesmus* we have found the following amino acids, listed in the order of decreasing relative intensity of ninhydrin color on the chromatogram: glutamic acid, "unknown,"[2] alanine, serine, arginine, valine, aspartic acid, leucines, phenylalanine, tyrosine, α-aminobutyric acid (?), lysine, β-alanine, threonine, glycine, and proline.[3] The radioactive amino acids photosynthesized by *Scenedesmus* from $C^{14}O_2$ in 30 sec (*1*) include predominantly aspartic acid[4] with somewhat less alanine. Other radioactive amino acids synthesized under these conditions and detected by radioautography included asparagine, serine, β-alanine, and phenylalanine.

When the radioactive amino acids synthesized in the dark (1 min) by preilluminated (10 min) *Scenedesmus* were separated, the predominant radioactive product was aspartic acid with somewhat less labeled alanine. Radioactive phenylalanine is synthesized in much smaller amount.

The analysis of *Chlorella* is not yet as complete as that for *Scenedesmus*. The following amino acids have been found in *Chlorella* extracts: glutamic acid, leucines, alanine, valine, glycine, and β-alanine. *Chlorella* which have been allowed to photosynthesize with $C^{14}O_2$ for 30 sec form a predominant amount of radioactive aspartic acid with almost as much alanine. Minor radioactive products include β-alanine and serine. Dark (1 min) $C^{14}O_2$ fixation by preilluminated (60 min) *Chlorella* yields largely radioactive alanine.

In all paper chromatograms the glutamic acid ninhydrin spot was strongly evident. In no case was any radioactivity found coincident with this spot. In cases where glutamine was present, no corresponding radioactivity was observed.

Thus, it appears that in both dark reduction of $C^{14}O_2$ and photosynthesis the same pattern of radioactivity in the amino acids occurs. In both cases the amino acids which have been identified correspond to the 3- and 4-carbon amino acids. This is in accord with the tentative scheme proposed earlier (*1*), which inferred that the 3-carbon amino acids, alanine, serine, and β-alanine, have their origin in pyruvic acid and the 4-carbon ones have the origin of their carbon skeletons in oxalacetic acid. The positive determination of the absence of radioactive glutamic acid is to be taken as evidence against the participation of the tricarboxylic acid cycle in the anabolic path of CO_2 in photosynthesis.

References

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[2] Identical with Spot #23 of Dent, Stepka, and Steward and very probably the same compound reported as (b) with chromatograms of *E. coli* digest (A. Polson. *Nature, Lond.*, 1948, **161**, 351).

[3] Due to the yellow color of this ninhydrin spot, it is not possible to compare its intensity.

[4] Aspartic acid may be as high as 75% according to co-crystallization assay.

Significance of Distribution of Fluorescein in Sarcoma 180[1]

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Moore, in a recent report (*2*) on the use of previously injected fluorescein in the diagnosis of human tumors at operation, stated that under the conditions of his study (dose—5 cc of 20% solution intravenously; duration between injection and operation—3–8 hrs) fluores-

[1] This work was conducted in part by a grant from the American Cancer Society to the Department of Preventive Medicine, The Johns Hopkins University School of Medicine, recommended by the Committee on Growth of the National Research Council. These studies form part of a joint project on the chemotherapy of cancer being conducted at The Johns Hopkins University School of Medicine, Department of Preventive Medicine, and the Medical Division, Army Chemical Center, Maryland.

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cence was found only in non-necrotic areas of either primary or metastatic tumors, although it also occurred in areas of edema or cyst formation. In experiments made to determine whether this report could serve as the basis for a method of determining viable areas of mouse tumors prior to transplantation, and thus of reducing the incidence of failure of take, the following results were obtained.

TABLE 1

DISTRIBUTION OF FLUORESCEIN IN SARCOMA 180 AT INTERVALS AFTER INTRAVENOUS ADMINISTRATION

Time	Fluorescence	
	Animal tissues*	Tumors†
Under 12 min	Marked	Nonuniform fluorescence, tending to be higher on periphery
12 min–3 hrs	Marked early to none at 3 hrs, with some variability	Uniform marked fluorescence
3–7 hrs	None	Marked fluorescence in necrotic areas: tended to decrease by 7 hrs

* Fluorescence was present at all times in the colon, gallbladder, and urinary bladder—the normal routes of excretion of fluorescein (*1*).

† In a few animals with spontaneous mammary carcinoma, this tumor showed essentially the same sequence of changes in distribution of fluorescein as the transplanted sarcoma 180.

Carworth Farm mice weighing 20–25 gm and bearing 15-day-old transplants of sarcoma 180 (with some variation in size) were injected intravenously with 0.25 cc of a 1:14 dilution of saturated fluorescein in normal saline and killed at various times after injection by crushing the cervical cord. The animals were opened, the tumors hemisected, and both animals and tumors examined under ultraviolet light. Sixty-eight mice (136 tumors) were studied in groups at intervals from less than 1 min after injection up to ½ hr, and at ½-hr intervals to 7 hrs. The findings in the various groups are summarized in Table 1.

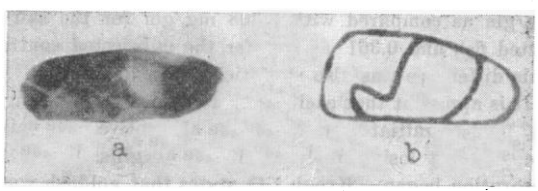

FIG. 1. (a) Section of tumor at 5 mμ; pale central area is necrotic. (b) Diagram of same tumor at autopsy; outlined central area showed fluorescence. Note that the necrotic and fluorescing areas correspond.

Microscopic sections of all tumors showed the presence of irregular areas of necrosis. Fig. 1b shows the area of fluorescence observed in one tumor; Fig. 1a, the section made from this tumor to show the essential identity of the area of fluorescence and the area of necrosis.

It is apparent from these data that the distribution of fluorescein in sarcoma 180 after a constant intravenous dose is determined by the length of time between injection and observation and by the amount of necrosis; at the longer durations the fluorescence was thus observed predominantly or only in the areas of necrosis. These results suggest that, in these tumors at least, a major factor determining the distribution of fluorescein in the tumors at any given time after injection is the slowness of diffusion into and out of poorly vascularized or necrotic areas.

Relative staining of viable and nonviable portions of tumors with fluorescein must thus be considered a dose-duration phenomenon, rather than the result of any specific staining potentiality of either portion.

References

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Physiological Adjustments in Chloride Balance of the Goldfish

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Absorption of certain ions, particularly chloride and sodium against an osmotic gradient, by the gills of certain fresh-water fishes was demonstrated by Krogh (*1*). The goldfish proved to be a favorable subject for these experiments, but in the case of chlorides, at least, Krogh (*2*) states that the body must be depleted below normal ion concentration of the internal medium before absorption occurs. He accomplished this by leaching the chlorides from the fish with distilled water. However, there are no quantitative studies showing that an initial reduction of the chloride level is necessary to evoke absorption of this ion. In the present studies data were obtained upon this phase of the chloride problem.

Vigorous goldfish (body weight, 45–95 gm) from a commercial hatchery which had been kept for at least 5 days without food in tanks of flowing water were used. Throughout the experiments each fish was held in a glass battery jar containing a carefully measured volume of tap water aerated continuously with washed, compressed air. In those situations in which it was necessary to separate the activity of the gills from that of the kidneys, a thin rubber sack was slipped over the tail of the fish and brought forward to the level of the pectoral fins, where it was bound snugly to the fish with cotton thread. Preliminary experiments had shown that this procedure did not interfere with equilibrium or locomotion of the fish. The urine and feces formed were collected in the rubber sack, but since the fish were in a fasting state, the material from the alimentary tract was negligible. The movements of the chloride ions were determined by analyses of the water surrounding the fish