coefficients. DNA, which is presumed to have a rigid two-stranded configuration in solution, has the lowest stacking coefficient observed. Heparin and polyphosphate, which are presumably flexible single stranded coils in solution have the highest stacking coefficients.

There is considerable evidence that Poly A undergoes a change in structure from a single-stranded coil above pH 7 to a rigid, two-stranded helix below pH 5. The stacking coefficient is high at pH 7 but low at pH 5 for Poly A. This gives further support to the idea that a high stacking coefficient corresponds to a flexible arrangement of binding sites, while a low one corresponds to a rigid arrangement, as found in a multistranded helix.

The structure of RNA is the subject of some controversy. Our sample has a stacking coefficient close to that of our sample of DNA, and we may infer that the two polymers have the same degree of molecular rigidity. Work is in progress to determine whether RNA and DNA samples from various biological sources, prepared by different methods, have similar stacking coefficients. Work is also in progress with metachromatic dyes other than AO. It has already been ascertained that the stacking coefficient of RNA, when measured with the dyes acridine yellow and coriphosphine O, is identical with that determined by means of the binding of AO.

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SEARCH FOR UNSTABLE CO₂ FIXATION PRODUCTS IN ALGAE USING LOW TEMPERATURE LIQUID SCINTILLATORS

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Introduction.—In early studies of photosynthesis in algae a variety of killing procedures was examined and compared. Since the various methods studied did not give very different results, killing with boiling ethanol followed by extraction with water was adopted as a standard procedure. In the work published in 1957—
A higher total fixation of C\textsuperscript{14} seemed demonstrable when photosynthesizing green algae (Scenedesmus or Chlorella) were killed by pouring their suspension into deeply cooled (−20° to −40°C) organic solvents (acetone or ethanol) than when they were killed by the usual procedure employing boiling ethanol. The percentage difference between the two killing methods (hot vs. cold) was greater in shorter periods of photosynthesis.

Preliminary killing experiments performed at temperatures between 0° and −70° showed that the most reproducible results were obtained between −20° and −40°. At temperatures near 0° one does not observe great differences from the normal procedure, and at the lower temperatures (−70°C) ice formation makes the manipulation difficult. A great part of the difference in the activity fixed in the cold and warm killing experiments could be caught as evolved CO\textsubscript{2} from the cold extract during manipulation. Because of what appeared to be suitable control experiments, this was attributed to the decomposition of an unstable compound different from bicarbonate, which could not survive plating or chromatography.

An attempt was made to learn more of this unstably bound CO\textsubscript{2} using the combustion method of Wilzbach and Sykes.\textsuperscript{a}

The Scenedesmus suspensions were poured into cold acetone or ethanol (−30°) and after addition of 1 ml of acetic acid for each 10 ml of original suspension to remove the excess of bicarbonate, an aliquot was taken by precooled micropipettes and injected into a very cold (−183°C/liquid oxygen) bomb with a copper-copper oxide mixture. After warming the tube to −45°, evacuating for a fixed short period of time, and sealing, the organic material was burned and the resulting radioactive carbon dioxide measured with a vibrating reed electrometer. By this method the "total fixation" was estimated. An equal aliquot spread on an aluminum plate and burned in a second bomb gave what was called the "stable fixation." The difference between the two measurements was attributed to "the unstable compound." The results were interpreted as confirmation of some of the differences described in the earlier work. No fixation was obtained in the experiments with the algae killed in acetone before the radioactive bicarbonate was added nor with the algae exposed to radioactive bicarbonate in the dark and flushed for 70 min with nitrogen. These results did not reveal the nature of the unstable compound or compounds, and in fact, left doubt as to their very existence.

For a more extensive examination of the unstably bound CO\textsubscript{2}, the determination of the radioactivity in liquid scintillators at −15°C seemed to offer an advantage over the very time-consuming combustion method of Wilzbach and Sykes. This method had been proved\textsuperscript{6} to give dependable results with the suspensions of inorganic compounds, homogenized tissues and bacteria.

Experimental.—Scintillation counting had never been used for the counting of algae suspensions, therefore, the following factors were studied before the method was applied to the low temperature killing experiments.

(a) the relative efficiency of this scintillation counting compared to that of Geiger-Mueller counting,

(b) the time course of the response of the scintillator containing a radioactivity sample (including bicarbonate),

(c) counting efficiency as a function of the algae content of the scintillator liquid,
(d) the effect of the temperature on counting. For the determination, 20 ml bottles were used containing as a scintillator 10 ml of 2,5-diphenyloxazole (PPO) solution in toluene (5 g in 1 kg). Three ml of absolute ethanol was added to each bottle to facilitate the solution of the aqueous suspension of algae. The liquid scintillation counter used in this study was a coincidence anticoincidence counter described earlier. The determinations were made at $-15^\circ C$. For the estimation of the relative counting efficiency of the scintillation counter with respect to the Geiger-Mueller counter, solutions of radioactive benzoic acid, sodium bicarbonate, and sucrose were used.

The results show that the counting in liquid scintillators offers a quick and suitable method for the estimation of the activity of the solutions of radioactive bicarbonate, benzoic acid, and sucrose in the presence of algal suspensions. The results are reproducible for several dilutions. The counting efficiency is 80 per cent for benzoic acid and sucrose. The activity of samples kept at $-15^\circ C$ does not change over a period of several hours. The apparent decrease in count caused by the presence of the algae suspension used in later experiments does not exceed 5 per cent.

This method was applied to the investigation of the possibility that the radioactivity found in previous experiments and ascribed to the presence of unstably bound CO$_2$ might be caused by a residue of the radioactive bicarbonate added to the suspension of photosynthesizing algae. To explore this possibility three kinds of experiments were done in which bicarbonate was added to:

1. normal photosynthesizing algae,
2. the algae kept in the dark,
3. the suspension of killed algae.

For these experiments three different post-killing conditions were applied for each of the two organic solvents;

(a) the suspension of algae was kept at $-45^\circ C$,
(b) the suspension of algae was kept at $-45^\circ C$ and nitrogen was bubbled through it for 15 min,
(c) the suspension of algae was kept at room temperature.

The conditions of the experiments were nearly the same as in the previous experiments except that the quantities used were only 1/10 as large. One ml of 2 per cent or 4 per cent suspension of Scenedesmus was preilluminated for 10 min in the presence of 1 per cent CO$_2$, then swept with N$_2$ for 3 min. Afterwards 4 or 8 $\mu$C of NaHC$^{14}$O$_{3}$ was added and the mixture was allowed to stand for 30 seconds. Then the algae were killed with acetic acid acidified solvents—4 ml of ethanol (for the 2 per cent suspensions) to give 80 per cent ethanol, or 9 ml of acetone (for the 4 per cent suspensions). A 4 per cent suspension of algae was used in the experiments with the acetone killing in order to produce a 0.4 per cent suspension of algae (as in the alcohol killing) in 90 per cent acetone solution. (The latter concentration was required because 80 per cent acetone freezes at $-45^\circ C$.)

In the cold killing experiments, (a) and (b), the algae were killed with the solvents cooled in liquid nitrogen to $-60^\circ C$ and placed immediately in an acetone-dry ice bath at $-45^\circ C$. In experiments (c) the killing was done with the solvents at room temperature. In all cases the 100 $\mu$ samples were taken as soon as possible; in (a) and (b) with pipettes precooled to $-45^\circ C$. In experiments (b) after the
taking of the first sample, nitrogen was bubbled through the suspension for 15 min.

Samples were taken every 15 min during the first 2 hours, the next one at 3 hours and another one the next day. The results of these experiments are represented in Figures 1–6. One can see that at room temperature bicarbonate has disappeared nearly completely after 1/2 hour from the ethanolic suspensions and somewhat more slowly from the acetone suspensions. The bubbling of N₂ for 15 min does not completely remove bicarbonate, although it diminishes its quantity. In ethanol solutions the differences between the radioactivity of the suspension of algae kept at -45°C flushed with nitrogen and that not flushed are much smaller than that...
in acetone solution. This is probably caused by the greater viscosity of alcoholic solutions at -45°C, from which CO₂ is not so easily expelled as from the acetone solutions which are completely liquid at that temperature. In the experiments in which the bicarbonate was added to the suspension of dead algae, one observes a complete disappearance of the radioactivity in ~12 hours. In the dark experiments in which the suspension of algae was flushed for 10 min with 1 per cent CO₂ and for 3 min in the dark with N₂, and the radioactive bicarbonate added for 30 sec before killing, one can observe a very small fixation.
Results.—Figures 1, 2, and 3 show that in the algae killed with alcohol and kept at −45°C practically all the bicarbonate disappears in 3 hours. This is not the case of acetone solution in which the radioactivity takes not less than 6 hours at −45°C to reach its final value (Fig. 4, 5, and 6).

Separate experiments not including algae showed that bicarbonate is considerably more stable in acetone than it is in alcohol at −45°C or lower. Beyond this the acetone volumes were larger than the alcohol volumes and the rate in bicarbonate escape was correspondingly slower.
Contrary to the previous results, it appears that in normal photosynthesis the stable fixation is the same no matter at what temperature the algae are killed. The results in Tables 1 and 2 show that in all cases the stable fixation measured in the scintillation and in the Geiger-Mueller counters was the same within the limits of accuracy of the methods.

These results fail to confirm the existence of an unstable product of early photosynthetic fixation persisting for some time at $-30^\circ C$ or $-45^\circ C$ different from and

more stable than bicarbonate. Our experiments also failed to confirm the differences previously found in the stable fixation of radioactive bicarbonate which appeared to depend on the temperature of the killing of photosynthesizing algae. On the contrary they proved that the stable fixation for the same suspension of algae for the same period of time, and the same quantity of added bicarbonate, is independent of the solvent used and of the temperature of the killing.

All the previous experiments can now be accounted for in terms of differences in the rate of escape of $C^{14}O_2$ from the various preparations. There remains the experiment in which the quantity of the apparent "unstably bound" $CO_2$ is dependent upon the immediate prior illumination of the algae (Expts. 2 & 7, Table 1, of Ref. 3). This seems now best interpreted in terms of a production of alkali as a result of the photosynthesis immediately preceding the administration of $C^{14}O_2$. Again independent experiments in the absence of alkali, with added quantities of alkali, produced the expected slowing of the escape of $C^{14}O_2$.

The possibility still remains that an "active" $CO_2$ may be present but in small amounts. This would be required if it is more or less stable than bicarbonate; if its stability is similar to that of bicarbonate these experiments could not distinguish it even in large amounts.

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RADIOACTIVE FALLOUT PARTICULARLY FROM THE RUSSIAN OCTOBER SERIES

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Introduction.—Radioactive fallout has been extensively studied and reported upon[12, 25, 29, 30] and, in general, although certain questions remain unanswered, the broad characteristics of its behavior have been established.

The stratosphere, the top fifth of the atmosphere lying above about 40,000 feet, plays an extremely important role. In fact, the fallout from megaton yield weapons occurs very largely from it while the troposphere is the medium which disseminates the fallout from kiloton detonations; thus, speaking broadly, stratospheric debris is from H-bomb detonations and tropospheric fallout is from A-bombs. It is not that the yield of the detonation is determinative, but rather that the altitude to which the fireball rises determines the fallout rates. The megaton yield fireballs are so enormous that they stabilize at levels only above the tropopause—the imaginary boundary layer dividing the upper part of the atmosphere, the stratosphere, from the lower part, the troposphere—while the kiloton yield fireballs stabilizes below the tropopause. The tropopause normally occurs at something like 35,000 to 55,000 feet altitude, although it depends on season and location. In other words, low yield bombs fired in the stratosphere would be expected to give the same fallout rates as high yield weapons do when fired in the troposphere—or on the surface. There is some small part of the fallout for megaton yield explosions which does not reach the stratosphere.

The stratospheric debris descends very slowly unless, of course, it is attached to particles so large as to fall in the first few hours, as in the case of surface bursts. This paper is concerned only with the worldwide fallout—that is, the fallout which does not occur in the first few hours—and excludes the local fallout which constitutes the famous elliptical pattern which is so hazardous because of the intensity of its radiation at early times but which, in test operations, is carefully restricted to test areas. It should be noted that the local fallout is a principal hazard in the case of nuclear war. Most serious attention should be paid to it in civilian defense programs.

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