TABLE 2

DETECTION OF RADIOACTIVE IMPURITY IN L-CYSTINE

Time	Radioactivity of solution*
hr	counts/sec/ml
3	2.6
6	2.9†
19	34.9

* The solvent was water at 0° C.
† After this measurement 15.1 mg of compound was added.

two flasks. After equilibration for 16 hr, 0.5-ml samples were withdrawn through pipettes fitted with cotton filters, and the radioactivities of the solutions were determined. To one flask was added 12.6 mg of the compound and equilibration was continued for another 24 hr. At this time measurements showed that the radioactivity of both solutions had remained constant.

The presence of impurities was demonstrated in the following experiment: A sample of chemically pure L-cystine was deliberately contaminated by crystallization from a solution containing a mixture of radioactive sulfur-containing compounds. The apparent specific radioactivity of the recovered cystine was 0.039 counts/sec/γ S. A sample of 1.5 mg of the radioactive compound was suspended in 6 ml of distilled water and samples were withdrawn after equilibration for 3 and 6 hr respectively. After 6 hr, 15.1 mg of the compound was added to the flask and an additional sample was taken after further equilibration for 19 hr. The increase in radioactivity of the solution after addition of more solid (Table 2) proved that the compound contained radioactive contaminants.

Reference

1. KAMEN, M. D. *U. S. Naval Med. Bull.*, Suppl., Mar.–Apr., 1948, p. 115.

Some Observations on Exchange of CO_2 Between $BaCO_3$ and CO_2 Gas[1]

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Previous studies on the exchange of CO_2 between $BaCO_3$ crystals and atmospheric CO_2 have shown that exchange takes place in the presence of moisture (*1*), and that the amount of exchange can be reduced by heat treatment of the $BaCO_3$ samples (*2*). An attempt has been made to confirm these results, and to obtain additional information on the factors which affect the amount of exchange, using C^{14}.

The original $BaCO_3$ had a specific activity of about 1.2×10^6 cpm/mg.[3] It was attempted to prepare an active $CO_3^=$ solution by heating this material with inactive $0.1N$ Na_2CO_3 for 1 week. However, no exchange occurred. Active $CO_3^=$ was then prepared by mixing active $BaCO_3$ with varying amounts of ordinary $BaCO_3$, evolving the CO_2 by treating with $3N$ HCl in a small glass generating apparatus, and passing the evolved CO_2 in a small stream of N_2 gas through a coiled glass tube filled with $3N$ NaOH solution. After absorption of CO_2, the NaOH solution was removed from the apparatus and diluted about threefold, and $BaCO_3$ was precipitated, in the cold, with $BaCl_2$ solution. This was then filtered and washed with water, alcohol, and finally acetone to hasten drying. The filter paper with the precipitate was pasted to aluminum disks for easier handling and to prevent curling upon drying.

The effect of different amounts of moisture on the amount of exchange was studied first. The method used

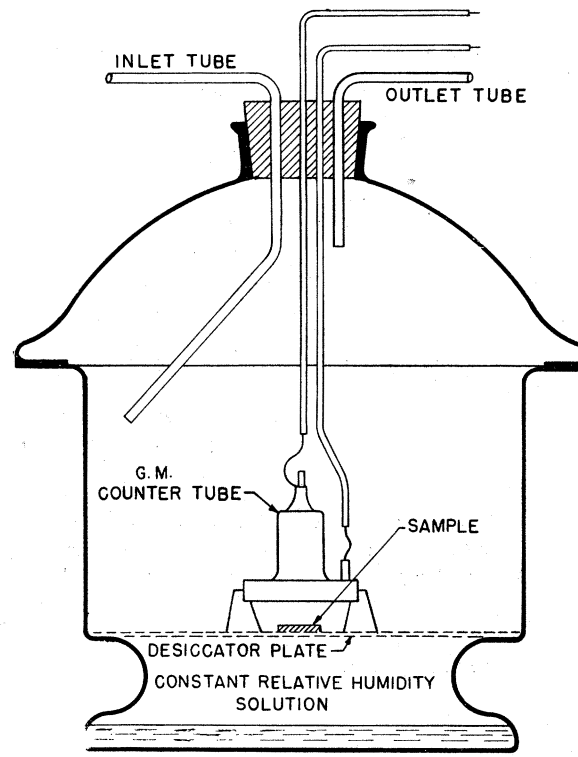

FIG. 1.

was to expose the sample to inactive CO_2 in a static atmosphere of known relative humidity, and to compare the counting rates of the sample before and after exposure. This was performed in a closed desiccator, as shown in Fig. 1. The bottom of the desiccator contained sulfuric acid solution of a density adjusted so that a known constant relative humidity was obtained.

The sample was placed beneath the tube and counted. CO_2 was passed into the desiccator for 15 min and then the vessel was sealed for 2 hr. At the end of this time, air was blown through to sweep out the CO_2, and the sample was recounted.

The addition of CO_2 into the desiccator causes the count to drop 25% to 40% of the count in air, due to increased absorption of β-rays by CO_2. However, all counting was done with air in the desiccator.

[1] Research work done at the Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.
[2] I wish to thank Dr. William Rubinson and Dr. Warren Miller for helpful advice.
[3] Obtained from Oak Ridge.

The results of the first set of experiments are given in Table 1. Each line represents a freshly prepared sample.

TABLE 1

Relative humidity in %	Wt of sample in mg	% Decrease in counting rate	
3.2	17.7	1.5	Prob. Error
3.2	15.8	1.2	
47.2	16.9	1.3	± 0.3%
47.2	17	1.3	
47.2	16.8	1.4	
75	17.3	1.4	
75	29.7	2.5*	
18.8	14.7	2.4*	± 0.5%
18.8	13.7	2.3*	
18.8	14.2	2.3*	

* Samples were prepared from a different active Na_2CO_3[4] solution.

The decrease in counting rate after exposure is taken to be a measure of the amount of exchange between CO_2 and $BaCO_3$. The results indicate that the amount of moisture present has no effect on the amount of exchange, at least so long as the relative humidity exceeds 3%.

Armstrong and Schubert (*1*) reported that thorough mechanical mixing of the barium carbonate after treatment caused no change in activity. This was interpreted as proof that exchange took place throughout the sample. This conclusion was confirmed by a different method, as follows.

A group of five samples was made up, each containing the same amount of activity, but varying in weight in steps up to 10 mg. Each sample was exposed to CO_2 in the manner already described, except that the CO_2 was moistened before being passed into the desiccator. The bottom of the desiccator contained water. After 2 hr the CO_2 was blown out and the sample was counted.

TABLE 2

Sample thickness mg/cm²	Loss of $C^{14}O_2$ in %	
0.56	3.8	Prob. Error
2.13	3.8	
2.59	3.8	
3.24	3.8	
4.44	3.9	± .5%
1.3	2.6*	
1.6	2.8*	
2.3	2.5*	

* Samples made from a new batch of Na_2CO_3 solution.

These results indicate nothing about the amount of exchange undergone by a single crystallite in the sample, but they do show that all crystallites in the body of the sample suffer exchange.

The following experiment was set up to determine the effect of heating on $BaCO_3$ samples. Yankwich (*2*) reported that heating $BaCO_3$ resulted in samples that could be stored for long periods without serious loss of activity.

Several samples were heated separately in air for ½ hr, 1 hr, and 1½ hr at 140° C. Each sample was then exposed to moist CO_2 for 2 hr and counted. Those samples which were heated for 1½ hr showed no exchange. Those which were heated for less time showed small, varying amounts of exchange.

TABLE 3

Preparation	Particle size	Exchange %	
Samples prepared by slow pptn. from hot soln.	Coarse crystals	0.0	
Samples pptd. at room temperature	Fine ppt., readily filterable	1.8	Prob. Error
		1.7	± .6%
Samples prepared from ice cold soln.	Fine ppt., clogged filter	4.5	
		4.5	

It was found that heating an active sample in air caused a loss of activity varying between 2.5% and 3%. However, when the samples are heated in an evacuated bulb, or in an atmosphere of nitrogen, this loss does not occur, and the sample is stabilized against subsequent exchange.

The next group of experiments was to determine what effect particle size had on the amount of exchange. Three sets of samples were made up. One set was made by dropwise precipitation over a 2-hr period from a very dilute solution kept at just below boiling temperature. Another was made at room temperature, and a third was made with a concentrated $BaCl_2$ solution from a semi-frozen solution of Na_2CO_3. Table 3 shows the results.

The final experiment was designed to get some idea of how the percent exchange depends on the time of contact. The rate of flow of CO_2 into the desiccator was kept as constant as possible from one sample to the next. After the CO_2 was blown into the desiccator for the time required the flow was turned off, and immediately air was passed through for 15 min. The samples for this experiment were precipitated from ice cold Na_2CO_3 solution to take advantage of the higher percent exchange which can be obtained by this procedure. The results are given in Table 4, and suggest that under these conditions, the exchange has almost reached its limiting value in 15 min.

TABLE 4

Time CO_2 was in contact with sample, in min	% Loss	
15	3.8	Prob. Error
15	4.1	
10	3.7	
10	3.2	
5	2.4	± .4%
5	2.3	
5	2.1	
2	2	
1	1	

The observations indicate that a small amount of moisture will be sufficient to cause exchange between $BaCO_3$ and atmospheric CO_2. However, the amount of

[4] All Na_2CO_3 mentioned in the article contains active carbon.

exchange taking place depends on the size of the particles; the larger the particles, the less the exchange. This indicates that exchange takes place on the crystal surface. Heating of the $BaCO_3$ sample or preparing it by some method giving very large crystals will make exchange negligible.

References

1. ARMSTRONG, W. D. and SCHUBERT, J. *Science*, 1947, **106**, 403.
2. YANKWICH, P. E. *Science*, 1948, **107**, 681.

Modifications of the Rabbit Ear Chamber Technique[1]

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The rabbit ear chamber provides a valuable technique for the microscopic study of living vascular tissue over a period of weeks or months. The basic design has been described (*1–3*), but in the course of the past three years various modifications have been made which simplify the procedure. Since the changes may be of help to others using the method, they are presented here.

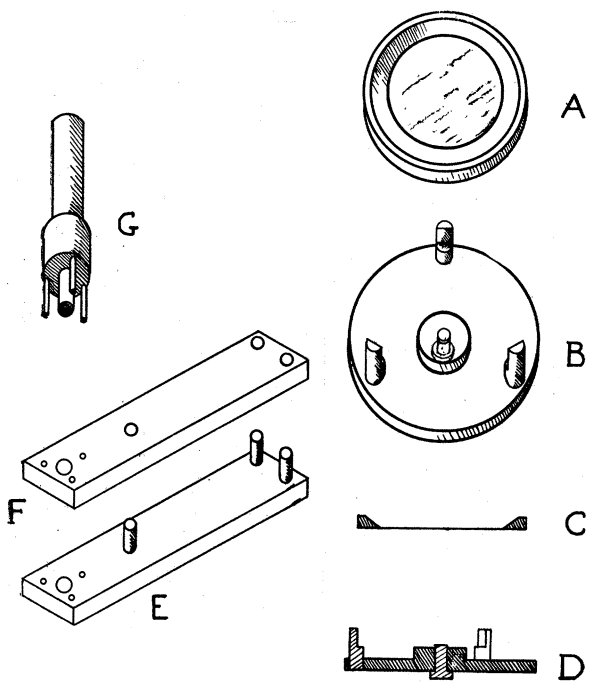

FIG. 1.

A modified chamber has been designed, constructed entirely of Plexiglas except for a mica cover slip. The cover slip's supporting ring (*A*) has an outside diam of $\frac{7}{8}$ in. and an inside diam of $\frac{5}{8}$ in. It is 0.080 in. thick. The top is beveled at the angle shown in cross section (*C*) to prevent high power objectives from striking the edge of the ring. A piece of clear mica 50–60 μ in thickness is glued to the supporting ring (*A* and *C*).

The chamber base (*B* and *D*) has a diam of 1 in. and the base plate (*B* and *D*) is 1/16 in. thick. A central observation table $\frac{1}{4}$ in. in diam projects 3/32 in. above the base plate. If access to the thin layer of tissue on the table is desired, a removable plug with a shaft diam of 1/16 in. may be made to fit loosely into a hole drilled through the base plate and central table (*B* and *D*). It is important to have this fit loosely, because serum which seeps around the plug makes it difficult to remove the plug after new tissue has grown onto the observation table.

The cover slip ring (*A* and *C*) fits tightly onto three notched Plexiglas pegs (*B* and *D*), 0.109 in. in diam, inserted at the periphery of the base plate and cemented with glacial acetic acid. The notches (*D*) are elevated .002 in. above the level of the table. The height of the notches above the table determines the thickness of the new tissue which grows between the cover slip and observation table and eliminates the need for buffers previously described (*1, 2*). After the ring has been snapped into place, it is secured by a drop of Lucite in chloroform over each peg.

The operative technique for inserting the chamber has been described (*1, 2*). To insert the chamber, four holes are punched through skin and cartilage near the tip of the pinna; a central hole to accommodate the central table and three peripheral holes for the pegs. A steel punch (*G*) has been designed to cut the four holes at one time and insure an exact fit. Three peripheral punches are attached to a handle in the exact positions of the Plexiglas pegs and made 0.015 in. larger than the diam of the pegs. A fourth punch, 0.015 in. larger than the central table diam, is attached centrally. The punches are $\frac{3}{4}$ in. long and have notched ends. A Plexiglas guide (*E, F*) permits visualization of vessels when holes are punched and keeps the ear perfectly flat. The guide is made in two pieces, each $5\frac{3}{4}$ in. in length, $1\frac{1}{4}$ in. wide, and $\frac{5}{8}$ in. thick. Steel connecting pins, $\frac{7}{8}$ in. long and 3/16 in. in diam, connect the two plates when in use. Punch holes are placed at one end and are made 0.001 in. larger than the diam of the corresponding punch. The end of the ear is slipped between the two plates and when the template has been placed so that the central artery is adjacent to the central hole and no major vessels will be cut by the punches, the plates are pressed tightly together. The guide is then held firmly against the table and the holes are punched.

[1] This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

References

1. EBERT, R. H., FLOREY, H. W., and PULLINGER, B. D. *J. Path. Bact.*, 1939, **48**, 79.
2. EBERT, R. H., AHERN, J. J., and BLOCH, R. G. *Proc. Soc. exp. Biol. Med.*, 1948, **68**, 625.
3. SANDISON, J. C. *Amer. J. Anat.*, 1928, **41**, 447.