some preparations, areas of radioactivity between organisms much smaller than the endoplasmic concentrations or the yeast cells were noted. It could not be decided whether these were foci of radioactive debris or organisms.

Since about 90% of the β-particles are absorbed by a thickness of 1.2 μ in a medium of unit density or by a thickness of 0.2 μ in a silver bromide medium, very few β-particles will affect more than two grains. This factor leads to increased resolution. We were unable to observe β-particle tracks from samples containing tritium. Some of the smaller individual yeast organisms gave radioautographic images 3 μ in diameter, and in some organisms extranuclear polar or peripheral concentrations of radioactivity as small as 0.5 μ were resolved. Resolution of less than 1 μ was readily discernible (Fig. 3). This observation was made in a radioautograph which contained a thin coating of Formvar over the emulsion. The image here was not as distinct as in some preparations, where peripheral concentrations were represented by a single-grain outline of the cell wall. Ectoplasmic concentrations of radioactivity in the paramecium disclosed that distinct images of 1-μ size were clearly resolved.

The usefulness of tritium, allied with radioautography, in tracer studies depends on the specific problem under study. Tritium atoms bonded stably to carbon can be used for the labeling of carbon atoms just as deuterium has been used (*16*). The preparation of certain tracer compounds using radiocarbon is sometimes very difficult. In these cases, it may be less difficult to introduce tritium by catalytic reduction of a related compound or isotopic exchange with the inactive compound. For example, the introduction of radiocarbon into the skeletal framework of a steroid compound is considerably more difficult, in general, than the introduction of hydrogen atoms bonded to carbon. Among the general synthetic methods may be included exchange between the organic compound and water, sulfuric acid or hydrogen gas containing tritium, the hydrogenation of a double bond, and biosynthetic techniques involving the culture of organisms in media containing tritium (*17*). The lability of hydrogen linked to oxygen and nitrogen in —OH and —NH_2 and to carbon atoms at sites of enolization must be kept in mind by the investigator.

Tritium has recently been made available by the U. S. Atomic Energy Commission in 100-mc and 1-curie lots at relatively small cost. Its half-life of 12.1 ± 0.5 years (*18*) is more than adequate for radioautographic studies. Its use in human beings requires information about the biological half-life and any specific sites of localization of radioactivity.

References

1. STEVENS, G. W. *Nature*, **161**, 432 (1948).
2. BOYD, G. A., and LEVI, H. *Science*, **111**, 58 (1950).
3. BOYD, G. A., *et al. Ibid.*, **108**, 529 (1948).
4. HARRIS, J., SLOANE, J. F., and KING, D. T. *Nature*, **166**, 25 (1950).
5. CAMPBELL, D. *Ibid.*, **167**, 274 (1951).
6. YAGODA, H. *Radioactive Measurements with Nuclear Emulsions*. New York: Wiley (1949).
7. CURRAN, S. C., ANGUS, J., and COCKCROFT, A. L. *Phil. Mag.*, **40**, 53 (1949).
8. WEBB, J. H. *Phys.. Rev.*, **74**, 511 (1948).
9. EIDINOFF, M. L., and KNOLL, J. E. To be published.
10. ———. *Science*, **112**, 250 (1950).
11. HILLIER, J. A., and GETTNER, M. *J. Applied Phys.*, **21**, 889 (1950).
12. SCHUTZ, A. S., and ATKIN, A. L. *Arch. Biochem.*, **14**, 369 (1947).
13. DONIACH, I., and PELC, S. R. *Brit. J. Radiology*, **22**, 184 (1950).
14. BEISCHER, D. E. U. S. Navy Proj., Rept. No. NM001059-16–04, U. S. Naval School Aviation Medicine, Pensacola, Fla. (1950).
15. KAPLAN, N. Reported by Yagoda [(*6*), 222].
16. KIMBALL, A. H. *Bibliography of Research on Heavy Hydrogen Compounds*. New York: McGraw-Hill (1949).
17. KAMEN, M. D. *Radioactive Tracers in Biology*. New York: Academic Press (1947).
18. NOVICK, A. *Phys. Rev.*, **72**, 972 (1947).

A Method for Initiating the Absorption of Carbon Dioxide during a Manometric Experiment

M. Rabinovitz[1] and John Ingraham[2]

Division of Biochemistry, Medical School, and Department of Botany, University of California, Berkeley

When the respiratory quotient of tissues respiring in the presence of carbon dioxide is determined, it is necessary to absorb this gas after the period of metabolism. Many techniques for accomplishing this (*1, 2*) require special glassware, such as the flasks designed by Dickens and Simer (*3*) or by Dixon and Keilin (*4*). Other methods (*2*), adapted for the generation of alkali in the side arm of a Warburg flask, require an extended period of time for the complete absorption of carbon dioxide. The method reported here permits the addition of strong alkali to a filter paper strip in the side arm of a standard Warburg flask with a minimum of special equipment. The alkali, thus distributed throughout the area of the filter paper strip, causes a rapid absorption of the carbon dioxide in the flask.

The apparatus consists of a stopcock (ST 7/22) mounted on a ground-glass connection which fits into the opening of a Warburg flask side arm (Fig. 1). The stopcock contains a well in the form of a groove with a capacity of 0.1–0.2 ml. The well and part of the reservoir may be filled with alkali by inserting the needle of a hypodermic syringe through the reservoir. Care must be taken that no air bubbles are trapped in the alkali of the well. The reservoir is then sealed with a small cork. The stopcock remains in the position for filling until the alkali is to be added to the flask. A strip of filter paper is then fitted into the ground-glass connection so that it touches the stopcock, the remainder of the strip projecting into the side arm of the flask. The apparatus is then inserted into the side arm.

Following a period of respiration, acid is tipped

[1] American Cancer Society fellow, upon recommendation of the Committee on Growth of the National Research Council.
[2] Atomic Energy Commission predoctorate fellow in the biological sciences.

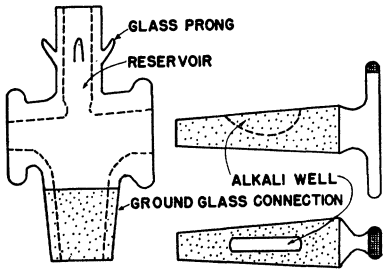

FIG. 1. Apparatus for addition of alkali to a filter paper strip in a Warburg flask side arm.

into the main compartment to stop metabolism and liberate bound carbon dioxide. The center well may be used for storing this acid. After the evolution of carbon dioxide is complete, the manometer is read and the stopcock turned 180 degrees until the alkali well touches the filter paper in the connection. The alkali will then wet the paper strip, absorb the carbon dioxide in the flask, and permit a manometer reading due to oxygen uptake alone. The respiratory quotient may be readily calculated from the two manometer changes and a knowledge of the volume of carbon dioxide originally present in the incubation medium.

For the calculation of volume changes from the manometer readings, it is necessary to use Warburg flasks calibrated with the apparatus inserted. The alkali well should not be included as part of the flask volume in this calibration. The reader is referred to the original papers (*3, 4*) and to standard texts (*1, 2*) for theory and applications.

References

1. DIXON, M. *Manometric Methods.* New York: Cambridge University Press (1943).
2. UMBREIT, W. W., BURRIS, R. H., and STAUFFER, J. F. *Manometric Methods and Tissue Metabolism.* Minneapolis: Burgess Pub. (1949).
3. DICKENS, F., and SIMER, F. *Biochem. J.*, **25**, 973 (1931).
4. DIXON, M., and KEILIN, D. *Ibid.*, **27**, 86 (1933).

The Thromboplastic Potency of Different Morphological Parts of Rabbit Brain

S. Gollub, F. E. Kaplan, D. R. Meranze, and David C. Schechter

Research Department,
Mount Sinai Hospital, Philadelphia

The thromboplastic materials currently used in blood coagulation studies are generally aqueous suspensions of dehydrated tissues and organs, principally brain, lung, and placenta. Ordinarily, entire organs are used for this purpose, obscuring the possible differences in thromboplastic potency between the morphological parts of the organ. It is the purpose of this paper to present data which indicate that the medullae of male rabbit brains differ significantly from the other morphological parts of the brain in thromboplastic potency.

The materials and methods have in the main been described elsewhere (*1*). For the purpose of this investigation the brain was divided into the following parts: cerebrum, cerebellum, corpora quadrigemini, cerebral peduncle, and medulla. In each experiment, the powders were prepared for extraction and suspension from the pooled similar parts of a dozen fresh rabbit brains.

Normal human oxalated plasma was used. The Quick one-stage method was employed with the following modification: suboptimal[1] concentrations of thromboplastic suspensions were used in order to slow the reaction by deficiency of thromboplastin. Variations in the thromboplastic potency would thus, we believe, tend to be more sensitively reflected in the clotting time.

TABLE 1

PLASMA CLOTTING TIME IN SECONDS WITH THROMBOPLASTIC SUSPENSIONS (0.1 G/10 ML PHYSIOLOGICAL SALINE) OF THE VARIOUS BRAIN PARTS

	Cerebrum	Cerebellum	Corpora quadrigemini	Cerebral peduncles	Medulla
Mean	19.4	20.1	22.1	19.6	33.0
Median	19.4	20.7	21.3	19.7	32.2
Low	16.4	13.3	16.5	15.2	20.4
High	22.2	32.3	34.2	24.3	47.9

The one-stage method was therefore used as a thromboplastin assay instead of a prothrombin test.

The acetone-dried residue of the rabbit brain had a mean dry weight of 1.2 g. Of this, the cerebrum, cerebellum, corpora quadrigemini, cerebral peduncle, and medulla represented 56%, 12%, 4%, 14%, and 12%, respectively. The powders were all cream-colored and had a faint characteristic odor. Insufficient trituration[2] yielded a cork-colored material with granular aggregates, and prolonged trituration produced a very fine, whitish powder. Both the latter products yielded much less active thromboplastic suspensions. The cause for this is not yet known.

Table 1 shows the summarized data of the clotting times of seven sets of preparations of the various brain parts, using 17 different normal plasmas. Each preparation represents the pooled similar parts of 12 rabbit brains. It is seen that the cerebral preparations were generally not only more active, but they also showed smaller variation than any other brain part. Statistical analysis (Student's "t" test) showed that the differences between cerebrum, cerebellum, corpora quadrigemini, and cerebral peduncle were not significant. There was, however, a highly significant difference between the medulla and the other brain parts as a whole ($p < .005$), as well as between the medulla and other brain parts individually.

It was of interest to determine whether the lower potency of thromboplastic suspensions from medulla

[1] By optimal concentration is meant that obtained from the smallest amount of powder/10 ml saline which will yield the shortest clotting time for normal plasma in the one-stage method. In our laboratory this is 0.5 g powder/10 ml saline. It clots plasma in about 11–12 sec. The suboptimal concentration used was 0.1 g/10 ml.

[2] In Waring blendor with acetone.