Technical Papers

Respiration of the Parts of the Hybrid Gastrula *Rana pipiens* × *R. sylvatica*[1]

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The general development of hybrid eggs obtained from the cross *R. pipiens* × *R. sylvatica* has been thoroughly studied by Moore (*1, 2*). He showed first of all that the hybrid eggs are blocked in most cases at a stage when a distinct dorsal lip is formed—that is, at stage 10 of Shumway (*3*)—and second, that the competence of the ectoderm and the inductive ability of the dorsal lip are generally weak in comparison with those of eggs from the cross *R. pipiens* × *R. pipiens*. The latter fact is especially interesting, since it suggests that the effect of hybridization may be a general disturbance of the eggs.

Barth (*4*) has found that the increase in respiration of hybrid eggs is also blocked beyond stage 10. The eggs continue to respire at the same rate as at the time when respiration is blocked. He has concluded that "not only does the dorsal lip metabolism remain at the gastrula rate, but all of the cells appear to continue to respire at this rate." Since the general respira- with those of eggs from the cross *R. pipiens* × *R. pipiens*) has already been studied by Sze (*5*), it was of interest to see whether the respiratory pattern of the hybrid gastrula is similar to that of the normal one.

Four regions of the hybrid gastrula, corresponding, respectively, to 1, 2, 3, and 4 in Fig. 1, were measured by the Cartesian diver technique. The results are given in Table 1. The average respiratory rates of regions 1, 2, 3, and 4 are 1.92, 2.25, 2.27, and 1.56×10^{-4} µl/µg/hr, with respect to dry weight, respectively. There is an apparent animal pole–vegetal pole respiratory gradient. Moreover, the dorsal lip in-

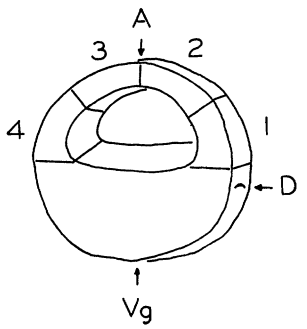

FIG. 1. Diagram showing the parts of the hybrid gastrula used in the measurements of the oxygen uptake. *A* = animal pole; *Vg* = vegetal pole; *D* = dorsal lip.

[1] This work was supported by a research grant from Committee on Growth acting for the American Cancer Society and administered by L. G. Barth.

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TABLE 1

RESPIRATION OF THE PARTS OF THE HYBRID GASTRULA, *Rana pipiens* × *Rana sylvatica*

Egg No.	Regions			
	1	2	3	4
1	1.71	1.48	1.96	1.46
2	1.57	2.57	2.36	1.41
3	2.27	2.77	2.74	1.81
4	2.04	2.15	2.23	1.60
5	2.00	2.26	2.08	1.51
Mean	1.92	2.25	2.27	1.56

Rate: $\times 10^{-4}$ µl/µg dry wt/hr.

variably has a higher respiration on the basis of dry weight than the ventral lip. When we compare the present data with data from the normal eggs of the same season as reported by Sze (*5*), we notice that the activities of the various regions of the hybrid eggs

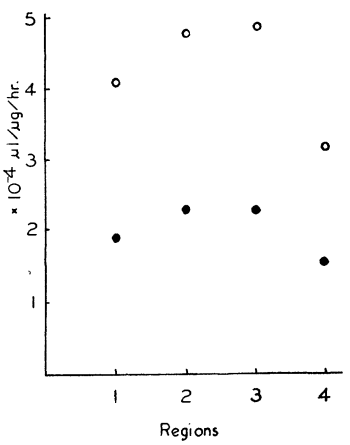

FIG. 2. Diagram showing the respiratory rates of the parts of the normal and hybrid gastrula. Hollow circles = normal; solid circles = hybrid.

are approximately half those in the corresponding regions of the normal eggs when their respiratory rates are expressed in terms of dry weight (Fig. 2). This indicates that the respiratory block in the hybrid embryo is not localized. Thus, the hybridization disturbs the respiration equally in every region of the egg.

It has been mentioned that the various regions of the hybrid eggs at stage 10 respire at only half the rate of the corresponding regions of the normal eggs taken at the same stage (on the same reference standard). The development of the hybrid eggs is slower, however, than that of the normal eggs. Usually, by the time the hybrid eggs reach stage 10, the normal eggs are already at about stage 11 of Shumway. Consequently, the above comparison is made on the basis of developmental stage. If we want to compare the relative rates of respiration of the parts of these two

groups of eggs on the absolute time scale, the normal eggs should be dissected at stage 10, and cultivated until the hybrid eggs of the same clutch are ready, and then measured simultaneously. One experiment has been carried out with region 1 as a test. This region of the normal egg was isolated first and cultivated under the same condition as the hybrid eggs. After the hybrid eggs reached stage 10, the corresponding region of the hybrid gastrula was dissected out. When the healing of the hybrid explant was complete, both the normal and the hybrid explants were measured simultaneously. The rates of the normal and the hybrid explants were, respectively, 4.17 and 1.60×10^{-4} $\mu l/\mu g/hr$ with respect to dry weight. The normal explant has a respiratory activity 2.6 times greater than the hybrid one.

The results of the experiments suggest that the effect of the *R. sylvatica* genome on respiration is widespread. There is no localized effect; that is, all cells are apparently affected similarly, since the general respiratory pattern of the hybrid gastrula remains the same as that of the normal gastrula, the only difference between these two kinds of eggs lying in the magnitude of the respiratory activity. These results are in agreement with the conclusion of Barth (*4*) and the results from experimental embryological studies of Moore (*1, 2*).

References

1. Moore, J. A. *J. Exptl. Zool.*, **105**, 349 (1947).
2. *Ibid.*, **108**, 127 (1948).
3. Shumway, W. *Anat. Record*, **78**, 139 (1940).
4. Barth, L. G. *J. Exptl. Zool.*, **103**, 463 (1946).
5. Sze, L. C. *Physiol. Zoöl.* (in press).

Manuscript received September 2, 1952.

Liquid Scintillation Counting of Tritium-labeled Water and Organic Compounds[1]

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The liquid solution scintillator is assuming an increasingly important role for homogeneous counting of C^{14} compounds. With such compounds as steroids, fatty acids, etc., which have little quenching action on the scintillation system, relatively large samples may be counted with high experimentally realizable efficiency (40–60%). The high efficiency, ease of operation, and the large range in sample size provide ample reason for establishing liquid scintillation counting as the method of choice in many biological experiments utilizing C^{14}. Solutions that have been employed for scintillation counting include *p*-terphenyl in toluene (*1*), 2,5-diphenyloxazole in toluene (*2*), and *p*-terphenyl in various mixtures of dioxane and water (*3*).

[1] Work done under the auspices of the Atomic Energy Commission.

[2] The authors wish to acknowledge the technical assistance of Betty Rogers, Kenneth C. Kohr, and Robert W. Merwin, Jr.

Instrumentation problems associated with liquid solution scintillation counting of low energy β-particles have been partly resolved by coincidence circuitry (*1, 2*) and refrigeration of photomultiplier tubes in order to lower the portion of the background counting rate that is due to dark current. The upper level discriminator operating in anticoincidence (*2*) has proved useful for rejecting high amplitude background pulses and improving the counting rate ratio of sample to background.

The considerably lower energy β-spectrum of H^3 relative to C^{14} indicates the probability of a correspondingly greater difficulty in realizing satisfactory counting conditions for tritium. The observation that tritium-labeled hexestrol (*4*) could be counted with low efficiency in the C^{14} scintillation system (*2*) suggested further investigation of this type of tritium counting.

Using the fast coincidence instrument designed for C^{14} scintillation counting, various tritium-labeled steroids and fatty acids were counted, and the counting efficiency was estimated by assay of the water obtained on combustion. A vibrating reed electrometer was used to compare the water with a standard tritium water sample (*5*). In addition, it was found possible to count tritium water directly with about the same sensitivity as with the electrometer, and with considerably greater rapidity and convenience.

2,5-Diphenyloxazole was employed as the solute with toluene, dioxane, and xylene as the major solvents. The standard practice was to use 100 mg solute in a total volume of 30 ml solution. Miscibility of tritium water with toluene or xylene was achieved by addition of absolute ethanol.

Contamination difficulties were minimized by use of Pyrex weighing bottles (45 mm in diam by 65 mm) as sample containers. These can be swiftly inserted into, and removed from, the area between the photomultiplier cathodes, thus permitting samples to be changed without warming of the tubes. The sample holders also may be easily cleaned or discarded when significantly contaminated.

Coincidence counting efficiencies reported here were accompanied by backgrounds of 100–200 cpm. Efficiencies from either one of the single tubes were 2–4 times higher than those from the two tubes operated in coincidence, but the backgrounds were of the order of 10,000–100,000 cpm.

Results obtained with a few sterols dissolved in toluene are given in Table 1. Samples ranging in size from fractions of a milligram to a gram may be counted with the same efficiency, the upper limit of sample size being conditioned only by the solubility of the compound. Although certain organic compounds exhibit a quenching effect, none of those reported in Table 1 showed perceptible quenching in the concentrations reported.

The counting data obtained for tritium water are presented in Fig. 1 and Table 2. The highest efficiency was realized with 0.01 ml water, 0.19 ml absolute