

ANAEROBIC AMMONIA PRODUCTION BY AMPHIBIAN GASTRULAE EXPLANTS

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Boell, Needham and Rogers reported (1939) that dorsal lip explants of *Rana temporaria* gastrulae, under anaerobiosis, produce some three times as much ammonia as ventral explants under similar conditions. Their finding is of considerable interest, for it is just this sort of metabolic correlate to gross morphology that chemical embryologists hope to discover. But attempts to confirm their results, together with a close examination of their experimental procedures, have convinced us that there is little basis for their claim.

NOTES ON METHODS

Explants: Dissections were made with glass needles in the usual manner. Explanted pieces were allowed to heal well in 100% Holtfreter's before beginning the experiment. Great care was taken to prevent contact of any explant with air-water interfaces—hence in no experiment was there any sign of cytolysis.

Weighing: Explants or brei (25–90 μg . dry weight) were pipetted on to small bits of cigaret paper, dried for one-half hour at 100° C., and weighed immediately on a quartz helix balance to $\pm 1 \mu\text{g}$.

Anaerobiosis: Explants or brei (ca. 1 mg. dry weight) were placed in 24–25 μl . of 100% Holtfreter's solution (with or without bicarbonate) in small aluminum-foil dishes. The dishes (blanks and experimentals) were set in a closed horizontal glass cylinder with a stopcock at each end. The gas mixture (95% N₂-5% CO₂) was run through the cylinder for a half hour after first being passed over a hot copper screen and through a water wash. (In two cases, hydrogen gas was used, de-oxygenated with hot platinized asbestos.) The stopcocks were then closed for the duration of the experiment (usually 3–4 hours). Under these conditions, methylene blue barely reduced with hydrosulfite did not change color for at least 18 hours; hence, anaerobiosis was at least as complete as in the experiments of Boell *et al.*, who used a similar criterion. Aerobic controls were kept in covered dishes in air.

Temperature: All experiments were conducted at room temperature (20–25° C.).

RESULTS

When whole gastrulae (*Rana pipiens*, stage 10, each weighing dry about 1 mg.) are cut into about 20 explants and placed at room temperature in Holtfreter's solution, they excrete traces of ammonia (all experiments were controlled with Holtfreter's blanks) into the surrounding medium:

(1) $0.45 \pm 0.21 \mu\text{g}$. NH₃/gastrula/5 hours (4 expts., aerobic).

Similarly treated gastrulae under anaerobiosis (*cf.* Notes on Methods) gave similar results:

(2) $0.20 \pm 0.17 \mu\text{g. NH}_3/\text{gastrula}/5 \text{ hours}$ (7 expts., anaerobic).

We do not attach any significance to these values other than that they indicate the excretion of small traces of ammonia. Our ammonia method (Brüel *et al.*, 1946) had an upper range of about $7 \mu\text{g. NH}_3$. Under our routine conditions, standard NH_3 samples are recovered with a reproducibility of about $\pm 2\%$ of the total range, *i.e.*, $\pm 0.14 \mu\text{g. NH}_3$. With this method, the ammonia present in the aliquots available for analysis—up to $0.13 \mu\text{g.}$, corresponding to that produced during the experimental period by about $300 \mu\text{g. tissue}$ —was barely detectable.

The results of Boell, Needham and Rogers are not much better off in this respect. From their account, it is easy to calculate that their ammonia method (similar to ours in all essential respects) had an upper range of about $3 \mu\text{g. NH}_3$. We shall make the fair assumption that the reproducibility of their determinations was $\pm 2\%$ of this range, *i.e.*, $\pm 0.06 \mu\text{g. NH}_3$. A few simple calculations from their data and the description of their experimental procedure show that in the case of the dorsal lip explants (their Table IV) the average aliquot actually taken for ammonia determination contained only about $0.18 \mu\text{g. NH}_3$, and that in the case of the ventral explants (their Table V) the average aliquot contained only about $0.075 \mu\text{g. NH}_3$. Since they must have been working almost at the limit of reproducibility of their method, it is clear that, like ours, their results:

(3) Dorsal lip: $1.75 \mu\text{g. NH}_3/\text{mg. dry wt.}/5 \text{ hours}$

(4) Ventral: $0.61 \mu\text{g. NH}_3/\text{mg. dry wt.}/5 \text{ hours}$

have little quantitative significance. ((3) and (4) have been recalculated from their tables so as to be comparable to our results (1) and (2).)

But to assure ourselves that we were not failing to confirm their results simply because our ammonia method was too insensitive, we scaled it down so that the upper range was approximately $1 \mu\text{g. NH}_3$. With this method, the anaerobic NH_3 production by pooled dorsal halves of 3 gastrulae was:

(5) $0.073 \mu\text{g. NH}_3/\text{mg. dry wt.}/5 \text{ hours}$;

for the corresponding ventral halves, the production was:

(6) $0.22 \mu\text{g. NH}_3/\text{mg. dry wt.}/5 \text{ hours}$.

Even with the scaled-down method the ammonia in the aliquots (corresponding to that produced by about $300 \mu\text{g. dry wt. tissue}$ —many times more tissue than employed by Boell *et al.*) was too low (0.017 – $0.068 \mu\text{g. NH}_3$) for accurate measurement; therefore neither the relative nor the absolute magnitudes of the values in (5) and (6) are to be taken seriously.

Nor does the difference between our claim and that of Boell *et al.* appear to be the outcome of studying gastrulae of different species: a dissected *Rana temporaria* gastrula put through our standard procedure failed to produce any ammonia anaerobically, and another failed to excrete any ammonia aerobically in four hours (in both cases, actually, the blank controls were slightly higher than the experimentals). (These gastrulae were kindly furnished us by Professor John A. Moore.)

Not only do gastrulae explants fail to excrete much ammonia into the circumambient medium, but they appear not to produce it *at all* in any significant amounts; for when explants (dissected whole gastrulae) at the end of the experimental period, whether aerobic or anaerobic, are ground up and precipitated with tungstate, the supernatant still contains only traces of ammonia:

(7) $0.50 \pm 0.11 \mu\text{g. NH}_3/\text{gastrula}/5 \text{ hours}$ (aerobic, 3 expts.);

(8) $0.30 \pm 0.11 \mu\text{g. NH}_3/\text{gastrula}/5 \text{ hours}$ (anaerobic, 3 expts.),

indicating that we do not fail to find ammonia in the medium around explants simply because the tissues bind it in some way as fast as it is produced.

But when *breis* are taken for ammonia analysis, large amounts of ammonia are obtained (presumably the strong alkali used in the method hydrolyzes ammonia-containing compounds in the tissues):

(9) $13.2 \pm 0.25 \mu\text{g. NH}_3/\text{gastrula}$ (3 expts.).

Now, (9) provides us with further grounds than those already advanced for casting grave doubt upon the significance of the claims of Boell *et al.* For it is quite likely that their explants underwent considerable cytolysis during their experiments (*cf.* Brachet's discussion of this point, 1950, pp. 375-376); and this means that in their analytical samples there must have been considerable ammonia-liberating cytolysate. We can understand, then, (a) why they seemingly found more ammonia to be excreted than they might have expected from their results on intact gastrulae (*cf.* their remarks: Boell *et al.*, 1939, p. 352); (b) why the dorsal explants seemed to produce more ammonia than the ventral explants, for it is well known that dorsal explants are more sensitive than ventral explants to all kinds of cytolysing influences (we have calculated on the basis of (9) that about an 8% cytolysis of their dorsal explants would have accounted for the reported difference from the ventral explants in NH_3 -production); and (c) why they found that NH_3 -production of dorsal tissue seemed to decrease with increasing age of their embryos (*cf.* their chart; Boell *et al.*, 1939, p. 348), for it is common knowledge that as embryos develop, their resistance to cytolysis in air-water interfaces increases tremendously.

Taken all together, the evidence almost forces us to conclude that although gastrulae explants excrete traces of ammonia both aerobically and anaerobically, these are so small as to be beyond the effective range of available ammonia methods; and that to date no quantitative statement about relative rates of ammonia excretion by different gastrula-parts has been experimentally justified. But if this is the case then the anaerobic glycolysis values of the Cambridge embryologists (Boell *et al.*) need re-evaluation also; for they were corrected for lactate allegedly bound by ammonia differentially excreted by dorsal and ventral explants.

LITERATURE CITED

- BOELL, E. J., J. NEEDHAM, AND V. ROGERS, 1939. Morphogenesis and metabolism: studies with the Cartesian diver ultramicromanometer. I. Anaerobic glycolysis of the regions of the amphibian gastrula. *Proc. Roy. Soc. London, Ser. B*, 127: 322-356.
- BRACHET, J., 1950. Chemical embryology. Interscience Press, New York.
- BRÜEL, D., H. HOLTER, K. LINDERSTROM-LANG, AND K. ROZITS, 1946. A micromethod for the determination of total nitrogen (accuracy $0.005 \mu\text{g. N}$). *Compt. Rend. des Trav. du Lab. Carlsberg, Ser. Chim.*, 25: 289-324.