to initiate the formation of antiprolan through protracted treatment and the capacity of paralyzing antiprolan *in vitro* is immanent in the active prosthetic group and not in the carrier substance which is hormonally ineffective.

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Metabolic Properties of the Regions of the Amphibian Gastrula.

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A number of authors have investigated the oxygen consumption and carbon dioxide production of the amphibian embryo at various stages of its development, with results of considerable interest. But such investigations throw no light upon the properties of the individual regions of the embryo at various stages, although the work of experimental embryologists has furnished us during the last 20 years with fundamental information about these regions and the part they play in the morphogenesis of the organism. In particular, the phase of gastrulation, during which are formed the germ-layers of classical embryology, and which involves the action of the primary organiser in determining the main axial structures of the embryo, merits the closest study.

Only recently have sufficiently delicate chemical methods become available for attacking this problem. Since Rehberg[1] developed the first ultra-micro burette, these methods have been greatly extended by the work of Linderstrøm-Lang, Holter, and their collaborators at Copenhagen. The first application of this kind of technic to the metabolism of the gastrula was made by Heatley,[2] who estimated the amounts of glycogen in the various regions of the gastrula and confirmed by direct chemical analysis the specially marked disappearance of this polysaccharide during the invagination of the roof of the archenteron, through the dorsal lip of the blastopore. All such observations have significance since it is in the dorsal lip of the blastopore and nowhere else during normal development, that the organiser "hormone" is liberated from its inactive combined form.

Wishing to study the metabolic properties of the dorsal lip of the blastopore, as opposed to the ventral ectoderm, where the organiser

1 Rehberg, P. B., *Biochem. J.*, 1925, **19**, 270.
2 Heatley, N. G., *Biochem. J.*, 1935, **29**, 2568.

is not normally liberated, by manometric means, we found in the Cartesian Diver ultra-micro-manometer a very suitable tool. The suggestion that the Cartesian Diver could be used for such a purpose we owe to Linderstrøm-Lang,[3] but he himself has not so far developed it for use with living tissues. If a bubble of air is enclosed in a small open glass bulb so that it floats within a larger vessel, the buoyancy of the diver will vary according to the pressure imposed on the whole system, and it will sink or rise as this pressure rises or falls. Conversely, if the gas phase in such a diver is increased or diminished by the process of a reaction within it, the pressure required to maintain it at a given level will correspondingly rise or fall. In this way the diver is equivalent to a constant-volume manometer, and we have found that a suitably modified formula of the Warburg type may be used for calibration, the volume of the diver being known. The divers used are readily made from Pyrex capillary tubing and consist of a neck, a bulb, and a tail to ensure that the diver floats upright. The neck varies from 6-16 mm in length according to the experiment which it is desired to make, and the total diver volume from 20-40 mm^3. It is desirable, in order that the loss of gas from the diver should be minimal, to use a strong salt solution as the flotation medium and since we wished to measure the ammonia produced during the experiment as well as the total nitrogen of the piece of tissue used, we substituted lithium chloride at the same density, for the saturated ammonium sulphate of Linderstrøm-Lang. Ammonia was estimated by the method of Linderstrøm-Lang and Holter[4] and total nitrogen by a new ultra-micro-Kjeldahl method devised by us (Needham and Boell[5]). The tissues used were for the most part single dorsal lips of gastrulæ and single pieces of ventral ectoderm, dissected out, often from the same embryo, by the Spemann glass needle technic Such pieces weigh less than 100 γ dry weight.

The delicacy of the method in its present form may be gauged by the fact that whereas 1 cm on the scale of the usual Warburg manometer corresponds to a gas change of about 20 mm^3, 1 cm on the scale of the diver manometer corresponds to a gas change of from 0.007-0.015 mm^3.

For the measurement of anaerobic glycolysis, the tissue lies in a film of Holtfreter-bicarbonate solution at the bottom of the bulb,

[3] Linderstrøm-Lang, K., *Nature*, 1937, **140**, 108.

[4] Linderstrøm-Lang, K., and Holter, H., *Comptes Rend. Trav. Lab. Carlsberg*, 1933, **19**, 1.

[5] Needham, J., and Boell, E. J., *Biochem. J.*, in press.

the gas space is filled with 95%N_2/5%CO_2 gas mixture, and the diver is sealed with the oil seal. Completely anaerobic conditions can only be attained by handling the divers within a special glass chamber with pipettes mounted on universal glass joints. A stream of the purified gas mixture passes through the chamber, and the divers are transferred from chamber to vessel carrying drops of lithium chloride solution which serve to protect them from the air during transit. The lithium chloride and the oil are saturated with the gas mixture beforehand. With these methods, an extensive set of measurements on the embryos of *Rana temporaria* showed a substantial difference between the organiser region and the ventral ectoderm.

	N_2/Q_L	NH_3 (cu mm $\times$ $10^{-3}/\gamma$ dw/5 hr)
Dorsal lip of blastopore	0.63	2.31
Ventral ectoderm	0.21	0.97

Another series of experiments on *Triton alpestris* gave a similar, though not quite so large, difference.

For the measurement of oxygen consumption, Holtfreter solution without bicarbonate was used and the neck of the diver was coated with paraffin wax. It was thus easy to suspend a drop of alkali at the lower end of the neck, just above the bulb, for the absorption of the CO_2 produced. Two series of experiments were performed, one on the embryos of *Discoglossus pictus,* the other on those of *Amblystoma mexicanum.* In neither case could any appreciable difference between the oxygen consumption of the dorsal lip and that of the ventral ectoderm be observed.

	Q'_{O_2} (related to total N, not dry wt)	
	Discoglossus	*Amblystoma*
Blastula roof	—	2.6
Dorsal lip of blastopore	4.8	3.2
Ventral ectoderm	4.8	3.2
Closing neural folds	3.0	—

For the measurement of respiratory quotient, a diver of larger volume and with a longer neck is used. The neck is paraffin waxed, as for oxygen consumption, but contains a series of drops or films in the following order: nearest the bulb, the drop of alkali, then a drop of acid sufficient to give a great excess of acid if mixed with the other solutions and tissue, then a further drop of acid in direct contiguity with the oil drop. At first the diver becomes steadily heavier owing to the absorption of oxygen by the tissue and of carbon dioxide by the alkali, but after a suitable time, usually here

3 hr, the system is subjected to a pressure of about a foot of mercury, which has the effect of blowing down the drops of alkali and acid into the bulb, the oil drop then returning to its original position, and the diver quickly becoming much lighter owing to the liberation of carbon dioxide. For the bound CO_2 of tissue and solutions a second diver is mixed at the beeginning of the experiment. The method is thus entirely analogous to that of Dickens and Simer,[6] who used annular cups on Warburg manometers.

As a result of experiments with this method, it seems that during gastrulation the dorsal lip of the blastopore shows a much greater trend towards unity than the ventral ectoderm. As was expected from the work of Brachet,[7] blastula roof gave a low quotient (0.75) and closing neural folds a high one (unity). Intermediate average values were as follows:

	Amblystoma mexicanum R.Q.
Blastula roof	0.75
¼-moon yolk-plug gastrula:	
Dorsal lip of blastopore	1.0
Ventral ectoderm	0.83
½-moon yolk-plug:	
Dorsal lip of blastopore	1.0
Ventral ectoderm	0.89
Closing neural folds	1.0

We are not yet in a position to say definitely whether the ventral ectoderm ever reaches a respiratory quotient of unity before it is completely underlain by invaginating mesoderm.

We should like to postpone the discussion of the meaning of these differences until the full publication of the data. In the meantime, we would draw the attention of biologists in general as well as embryologists to the existence of the very delicate and reliable ultra-micro-manometer now available in the Cartesian diver.

[6] Dickens, F., and Simer, F., *Biochem. J.*, 1930, **24**, 905.

[7] Brachet, J., *Arch. Biol.*, 1934, **45**, 611.