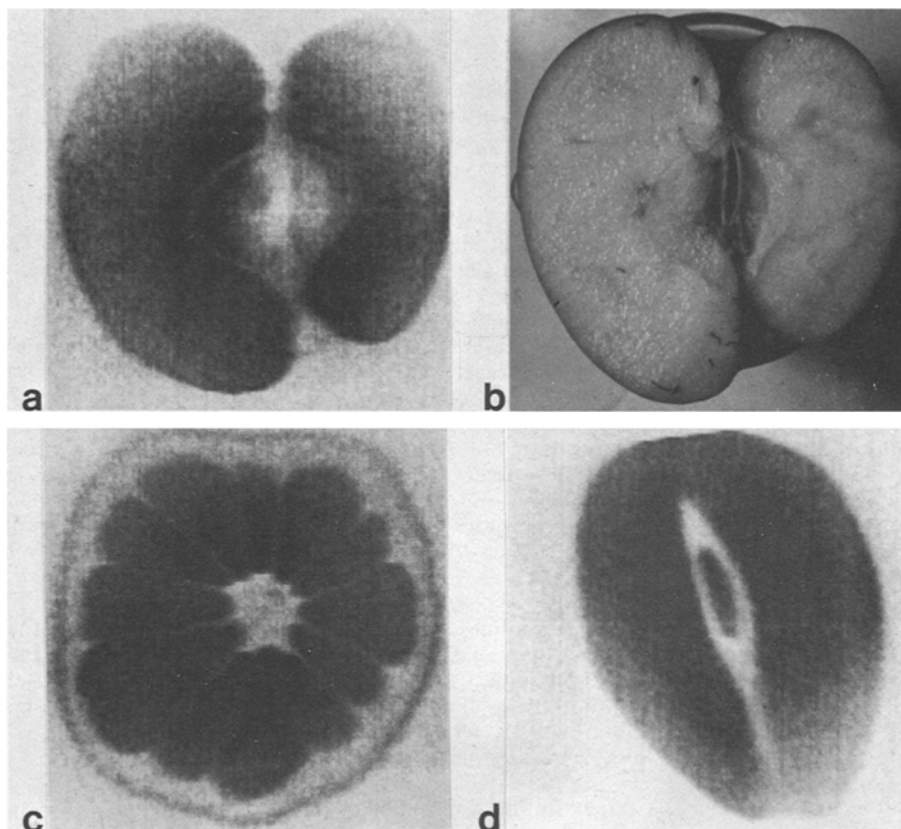


a, *c* and *d* show thin section NMR images of intact apple, satsuma and plum respectively. *b* is a subsequent section taken at the level of the imaging plane of the apple. These images demonstrate the resolving power of NMR imaging when applied to biological systems.



images of biological objects are produced in a reasonable time. Tissue contrast is clearly demonstrated and can be attributed to variations in water content and NMR relaxation times, signal amplitude increasing as the mobility of the system increases¹¹. Further exploitation of the method could involve such intriguing possibilities as the measurement of NMR relaxation times, diffusion coefficients, flow, chemical shifts¹², and the distribution of other resonant nuclei^{13,14} at localized regions within intact heterogeneous systems.

- 1 Acknowledgment. We thank Professor E.R. Andrew, Dr W.S. Moore and Miss C. Simaraj for their contributions. The work was supported by MRC grant G975-102.
- 2 Present address: Department of Radiology, Massachusetts General Hospital, Boston (Massachusetts 02114, USA).
- 3 Present address: Department of Physiological Chemistry, Johns Hopkins University School of Medicine, 725 N Wolfe Street, Baltimore (Maryland 21205, USA).

- 4 P.C. Lauterbur, *Pure appl. Chem.* **40**, 149 (1974).
- 5 W.S. Hinshaw, P.A. Bottomley and G.N. Holland, *Nature* **270**, 722 (1977).
- 6 P. Mansfield and I.L. Pykett, *J. Mag. Res.* **29**, 355 (1978).
- 7 P.A. Bottomley and E.R. Andrew, *Physics Med. Biol.* **23**, 630 (1978).
- 8 W.R. Inch, J.A. McCredie, R.R. Knispel, R.T. Thompson and M.M. Pintar, *J. nat. Cancer Inst.* **52**, 353 (1974); J.C. Eggleston, L.A. Saryan and D.P. Hollis, *Cancer Res* **35**, 1326 (1975).
- 9 W.S. Hinshaw, *J appl. Phys.* **47**, 3709 (1976).
- 10 E.R. Andrew, P.A. Bottomley, W.S. Hinshaw, G.N. Holland, W.S. Moore and C. Simaraj, *Physics Med. Biol.* **22**, 971 (1977).
- 11 W.S. Hinshaw, E.R. Andrew, P.A. Bottomley, G.N. Holland, W.S. Moore and B.S. Worthington, *Br. J. Radiol.* **51**, 273 (1978).
- 12 P.C. Lauterbur, D.M. Kramer, W.V. House and C.N. Chen, *J. Am. chem. Soc.* **97**, 6866 (1975).
- 13 D.I. Hoult, *J. Mag. Res.* **26**, 165 (1977).
- 14 G.N. Holland, P.A. Bottomley and W.S. Hinshaw, *J. Mag. Res.* **28**, 133 (1977).

Methods of experimental yolk removal from *Brachydanio rerio* eggs

Eva-Maria Langer

Zoologisches Institut der Westfälischen Wilhelms-Universität Münster, Hüfferstrasse 1, D-4400 Münster (Federal Republic of Germany), 27 November 1978

Summary. The yolk of *Brachydanio rerio* eggs was removed by pipettation, bursting and cutting off with a scalpel. The total yolk removal before the 8-cell stage led to a germ with irregular groups of cells, at the 8-cell stage to a topologically irregular differentiation, at the 64-cell stage to a nearly normal embryo, which is not viable.

The influence of yolk on the development of the extreme yolky teleostean egg with partial egg-cleavage of *Brachydanio rerio* can be investigated. 3 methods of experimental yolk removal will be demonstrated. The standard develop-

ment of the zebrafish was described in detail by Hisaoka and Battle¹. Experimental yolk removal from other teleostean eggs was carried out by Oppenheimer² and Trinkaus and Drake³ in *Fundulus*, Tung and Tung⁴ in *Carassius*,

Devillers⁵ in *Salmo* and *Esox*, and Kastomarov⁶ in *Misgurnus*.

Methods. Eggs of *Brachydanio rerio* are relieved from chorion by means of fine dissecting needles and brought into sterile, lid-covered glass vessels filled with Holtfreter's solution. The following methods of experimental yolk removal have proved appropriate.

1. Partial yolk removal by the aid of a pipette. Half of the yolk is removed by pipettation at the 64-cell stage and at blastula stages. The pipettes are made of thin glass tubes of an outer diameter of 3 mm and a wall-thickness of 0.5 mm. The diameter of the pipette opening is 50 μ m. A smaller diameter proves inefficient when sucking up the relatively large yolk granules. The pipette, inserted into the vegetative pole of the egg, sucks up the yolk by capillary attraction. After the pipette has been carefully removed from the yolk, the wound is immediately closed by the yolk membrane, which prevents a further loss of yolk.

This method turned out to be unsuitable for total yolk removal, because a removal in this way is impossible without severely damaging the blastoderm. Moreover, young cleavage stages can only be treated with a great loss of material.

2. Total yolk removal by bursting. Total yolk removal is possible by utilizing the surface tension of the rearing solution. The egg, freed from chorion, is brought to the surface of the liquid by sucking it slightly into a Pasteur pipette. Once in contact with the surface of the liquid, the surface tension tears the yolk membrane, which makes the yolk float out. The blastodisk rounds off and sinks to the bottom of the glass.

This method represents a rather simple way of removing the yolk totally. But in contrast to method 1 the yolk membrane is also removed. That, however, does not prevent a further development. Unfortunately this method is only applicable to blastula stages, because the cells of the blastodisk of young cleavage stages are damaged by the surface tension of the liquid.

3. Yolk removal by cutting it off with a scalpel. Yolk removal by cutting it off with a scalpel can be carried out on blastula stages as well as on young stages. The yolk is cut off below the margin of the blastodisk. The relatively large wound closes quickly. As the blastodisk lies like a cap on the yolk sphere, often the total yolk cannot be removed by 1 cut, which results in the remaining of considerable yolk quantities in the egg. After a 2nd cut, however, the wound is often so large that the egg dies.

This method of yolk removal can certainly be carried out quickly, but it is accompanied by a considerable waste of material.

Results. If the yolk is totally removed before reaching the 8-cell stage, there arise germs with irregular groups of cells, which cannot be related to a certain germ layer. If the yolk is removed completely from the 8-cell stage, an irregularly shaped germ develops, which has a chorda, nerve-tissue and somites. Entodermal derivatives cannot be observed. The tissues do not show any topological order and are to be found among cells, which remained undifferentiated.

Germs, developing without yolk from the 64-cell stage on, grow for about 50 h without retardation into almost normal embryos. After 70 h a further development cannot be observed; 30–40 h later the germs dissolve.

If only half the yolk is removed from the 64-cell stage, for 150–200 h the egg develops without retardation into an embryo whose trunk-tail-region is bound dorsally. The head is well developed. But oedema is to be found in all tissues.

- 1 K.K. Hisaoka and H.I. Battle, *J. Morph.* 102, 311 (1958).
- 2 J.M. Oppenheimer, *J. exp. Zool.* 72, 247 (1936).
- 3 J.P. Trinkaus and J.W. Drake, *J. exp. Zool.* 132, 311 (1956).
- 4 T.C. Tung and Y.F.Y. Tung, *Proc. zool. Soc. Lond.* 114, 46 (1944).
- 5 C. Devillers, *Experientia* 3, 71 (1947).
- 6 A.A. Kostomarov, *J. Embryol. exp. Morph.* 22, 407 (1969).

Computer-compatible system for continuous volumetric measurement of water consumption¹

W. Classen, J.R. Martin and K. Bättig

Institut für Verhaltenswissenschaft, Eidgenössische Technische Hochschule, Turnerstrasse 1, CH-8092 Zürich (Switzerland), 19 April 1979

Summary. An inexpensive drinkometer system is described that is compatible with most data acquisition systems and can be used for continuously monitoring the drinking pattern of laboratory animals. The change in electrical inductance resulting from the descent of a free-floating ferrite unit through a drinking tube, surrounded by a double coil, is converted to a signal proportional to the volume of fluid consumed.

A number of diverse techniques have been devised to continuously monitor water consumption. Typically, the number of licks has been measured^{2,3}. However, such systems accurately indicate the volume of fluid consumed only when the lick volume remains relatively constant. Although it has been reported that the volume consumed per lick is invariable⁴, other studies have noted considerable variation between animals⁵. Furthermore, alterations

in the feeding schedule can affect lick volume⁶ and the volume per lick has been found to vary by as much as 50–70% in individual rats from one day to another during the acquisition of schedule-induced polydipsia in this laboratory⁷. It is also questionable whether the constancy of lick parameters extends to brain-damaged or drug-treated animals exhibiting impaired motor coordination or stereotypic responses. In addition, those lickometer circuits that are

Drinking parameters for vagotomized and control rats

Parameter	Control (n = 4)		Vagotomized (n = 6)	
	Night	Day	Night	Day
12-h intake (ml)	30.7 \pm 2.4	2.6 \pm 0.7	8.8 \pm 4.2**	8.5 \pm 4.3*
Bout size	1.7 \pm 0.4	1.4 \pm 1.1	2.4 \pm 1.7	2.0 \pm 0.9
Number of bouts	18.7 \pm 3.2	1.7 \pm 0.6	5.2 \pm 3.9**	4.0 \pm 1.1**

Note: all values are mean \pm 1 SD. ** $p < 0.01$, * $p < 0.05$; compared to control group with nondirectional Student t-test.