Figure 3 also shows an alternative arrangement for the drive to the outer shaft and for the ducts in the inner shaft. Either arrangement of drive and ducts, as shown in figures 2 and 3, can be combined with either type of baffle plate.

Comparison of rotator and roller systems. The table shows the results obtained in an experiment to compare the rotator system with a roller system employing sealed bottles. All 8 bottles were of the type and size (capacity 30 ml) shown in figure 2 and each contained 5 ml of rat serum. 4 of the bottles were incubated on a rotator as in figure 2 with a continuous supply of 20% O_2/5% CO_2/75% N_2. The other 4 bottles were gassed initially with the same gas mixture and then stoppered and incubated on motor-driven horizontal rollers. 2 of the bottles in each group contained rat embryos (5 embryos per bottle) explanted at the early somite stage (10.5 days gestation) by methods described previously[12].

After 24 h incubation, all the embryos had developed normally to early limb-bud stages with a good heartbeat and blood circulation. The total protein content of the 5 embryos (with their membranes) in each bottle increased from about 0.5 mg initially to about 2.5 mg at the end of the 24-h culture period. There was no significant difference between the embryos grown in the rotator bottles and those in the roller bottles, but differences were found in the pO_2, pCO_2, pH and osmolarity of the culture serum.

pO_2. Without embryos, the serum in the roller bottles showed a higher pO_2 than in the rotator (as would be expected, because the roller bottles were gassed and stoppered at room temperature, before incubation). But with embryos present, pO_2 in the roller bottles fell to well below that of the rotator bottles.

pCO_2 and pH. Without embryos, pCO_2 was lower, and pH correspondingly higher, in the serum in the roller bottles than in the rotator, presumably as a result of different equilibria established between dissolved CO_2, carbonates and bicarbonates. With embryos present, pCO_2 more than doubled in the roller bottles but remained constant in the rotator bottles; in both systems there was a fall in pH, but the fall was much greater, to about 6.95, in the roller bottles.

Osmolarity. The osmolarity of the serum in the rotator bottles was slightly lower (about 295) than in the roller bottles (about 300), probably because the gas flow to the rotator was humidified by bubbling through distilled water, resulting in a small net transfer of water vapour to the serum. The effect is probably insignificant for tissue growth, but could be avoided by humidifying the gas with a salt solution instead of distilled water.

Discussion. In the experiment described, tissues (i.e. somite-stage rat embryos) were chosen which grow equally well in both rotator and roller systems and could therefore be regarded as providing comparable levels of O_2 consumption and CO_2 production in each system. The results show that in the rotator system, pO_2 and pCO_2 in the culture medium remain constant, while in the sealed roller bottles, pO_2 and pCO_2 may undergo large changes. Furthermore, the fall in pH in the rotator bottles is much less than in roller bottles. For cell, tissue, organ and embryo cultures where pO_2, pCO_2 and pH levels are critical, and where it is necessary to define these levels closely, the rotator system with a continuous gas flow has clear advantages.

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2 J. Paul, Cell and Tissue Culture, 5th ed. Livingstone Ltd, Edinburgh and London 1975.
3 G.M. Hodges, in: Organ Culture in Biomedical Research. Ed. M. Balls and M. Monnikendam. Cambridge University Press, 1976.
4 C.R. Austin, ed. The Mammalian Fetus in vitro. Chapman and Hall, London 1973.
5 D.A.T. New, P.T. Coppola and S. Terry, J. Reprod. Fert. *35*, 135 (1973).
6 D.M. Kochhar, Teratology *11*, 273 (1975).
7 N.D. Agnish and D.M. Kochhar, J. Embryol. exp. Morph. *36*, 623 (1976).
8 E.M. Deuchar, J. Embryol. exp. Morph. *35*, 345 (1976).
9 D.A.T. New, P.T. Coppola and D.L. Cockroft, J. Embryol. exp. Morph. *36*, 133 (1976).
10 M.K. Sanyal, 1978.
11 S.K.L. Buckley, C.E. Steele and D.A.T. New, Devl Biol. *65*, 396 (1978).
12 D.A.T. New, in: Methods in Mammalian Embryology. Ed. J.C. Daniel. W.H. Freeman & Co. 1971.

PRO EXPERIMENTIS

Immunological tests for LH and FSH in urine

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Summary. The addition of 100 g/l NaCl and 200 mg/l nitrofurantoin to 24-h urine samples destined for determination of LH and FSH with haemagglutination tests, proved to be an adequate preservative in comparison to chilling.

Immunological methods for detecting FSH and LH are relatively new. Haemagglutination inhibition (HAI) tests[1-3] are easy to perform, using erythrocytes sensitized with FSH or human chorionic gonadotrophin (HCG), respectively. Such erythrocytes may be agglutinated by an antiserum against FSH or HCG. Agglutination may be prevented by free FSH or HCG and, in the latter case, also by LH, which cross-reacts with the antiserum against HCG. Since HCG is found only in the urine of pregnant women and in some rare diseases (e.g. chorionepithelioma), the latter test may serve to determine LH in all other cases.

The HAI tests are designed to analyse urine, which, if the test cannot be carried out immediately after urination, should be kept in cold storage, or preferably frozen. Urine should not be kept in cold storage longer than 24 h. In view of the practical drawbacks of this method of preserving urine, we have looked for an alternative method, i.e. the addition of suitable preservatives. Substances with an aro-

Table 1. Results of LH and FSH tests carried out on 24-h urine samples with and without added preservative (nitrofurantoin 200 mg/24 h + NaCl 100 g/24 h)

Initials	Age	LH in IE/24 h		FSH in IE/24 h	
		Without preservative	With preservative	Without preservative	With preservative
H.v.R.	20	10	–	5	–
		13	25	13	3
L.v.G.	20	23	–	11	–
		21	24	10	12
R.J.L.	24	9	10	9	5
R.R.	26	15	23	4	3
R.v.d.B.	27	7	21	7	5
J.v.d.S.	27	12	–	12	–
		12	6	12	1½
W.K.	31	19	–	19	–
		15	20	15	5
J.t.H.	32	7	42	8	0
v.B.	38	4	26	8	13
P.St.	39/40	8½	–	11	–
		19	16	19	4
N.K.	42	12	14	6	2
K.H.	42	23	32	23	8
H.W.	46	7	16	3	3
P.J.S.	47/48	9	–	9	–
		27	20	27	10
W.v.S.	47/48	17	49	9	6
B.H.B.	51	259	11	15	3
L.v.G.Sr.	51	21	–	5	–
		11	32	11	16
P.v.R.	54	298	–	37	–
		41	36	41	9
C.B.	54	16	28	16	7
H.J.	60	10	12	10	12
Range		4–298	6–49	3–41	0–16
n		28	20	28	20

n = result not available

Table 2. FSH and LH values in the urine of healthy men (10th to 90th percentile) obtained in various centres

		Preservation	LH (IE/24 h)	FSH (IE/24 h)
National Institute	(n = 28)	None	8.5–27	5–23
	(n = 20)	Nitrofurantoin + NaCl	11–36	2–12
9 other centres	(n = 134)	Refrigeration	9–40	2–12

matic ring system are obviously excluded as they may interact with the reagents. Suitable preservatives should meet the following requirements: a) They should prevent or at least greatly prevent bacterial growth. b) They should not interfere with the immunological tests. In this respect, substances which have a low electroviscous effect (e.g. NaCl) are to be preferred. c) They should be stable and cheap.

On testing a number of preservatives, it was found that a combination of NaCl (100 g/l or 24-h urine) and nitrofurantoin (200 mg/l or 24-h urine) gave the best results. The others, Tego 51/15 DL®, streptomycin, oxolinic acid and thiomersalate, all interfered with one or both immunological tests. In a concentration of 200 g/l NaCl caused interference in 1 out of every 10 FSH tests and this made us decide to reduce the concentration to 100 g/l.

To validate this method of conservation, 24-h urine samples were collected of 20 healthy male volunteers to be tested with the above-mentioned HAI methods on LH and FSH. Each volunteer supplied 2–3 24-h urine samples. No refrigeration was used; one 24-h sample of each volunteer was collected in a bottle containing 100 g NaCl and 200 mg nitrofurantoin. FSH and LH tests in 24-h urine samples were carried out immediately after the collection was completed.

In table 1 the results obtained with the HAI tests are presented. Subsequently our results were compared with results obtained in 9 centres, where conservation of the urine by refrigeration is practised (table 2).

The immunological results obtained from the samples that had been preserved with NaCl and nitrofurantoin corresponded to those obtained from chilled samples, but those from unchilled unpreserved samples differed with regard to both FSH and LH.

We therefore concluded that the use of suitable preservatives is an acceptable alternative to chilling. It should be stressed that the urine should be collected in bottles already containing the preservative, or failing that, should be transferred to such bottles immediately after urination.

1 A.H.W.M. Schuurs and C.J. van Wijngaarden, Acta endocr. (Khb), suppl. *141*, 13 (1970).
2 A.H.W.M. Schuurs and C.J. van Wijngaarden, J. clin. Endocr. Metab. *50*, 619 (1975).
3 A.H.W.M. Schuurs, C.J. van Wijngaarden, J. Kačaki and S.M. Stulemeyer, personal communication.

CORRIGENDUM

H. Anhut, B.A. Peskar, W. Wachter, B. Gräbling and B.M. Peskar: *Radioimmunological determination of prostaglandin D_2 synthesis in human thrombocytes,* Experientia *34*, 1494 (1978). The legend for figure 1 (page 1495) should correctly read: Release of TXB_2 (O——O, right ordinate) and PGD_2 (●——●, left ordinate) from washed human thrombocytes at 37 °C after addition of thrombin. The results represent the means ± SEM of 5 experiments.

CURSUS

USA

6th summer program in methods of immunologic research and diagnosis

Buffalo, 4–22 June 1979

The sixth biennial program on current methods of immunologic research and diagnosis will be offered by The Center for Immunology of the State University of New York at Buffalo June 4–22, 1979. It will consist primarily of a combination of core and elective practical laboratory exercises to allow the participant to become acquainted with the techniques by personally carrying them out at the bench complemented by demonstrations, lectures and discussions. The entire program is designed to provide the participant with a survey of presently-available methodology and insight into the underlying immunologic principles. Further information may be obtained from James F. Mohn, Director, The Center for Immunology, State University of New York at Buffalo, 210 Sherman Hall, Buffalo, New York 14214. The deadline for applications is 31 March, 1979.

CONGRESSUS

Switzerland

3rd European symposium on vitamin B_{12} and intrinsic factor

Zürich, 5–8 March 1979

The symposium will be devoted to 1. Chemistry and biochemistry of corrinoids, and 2. Medicine, physiology and microbiology of corrinoids. Information from the organizing committee: Doz. B. Zagalak, Chemisches Laboratorium, KISPI, Univ. of Zürich, Steinwiesstrasse 75, CH-8032 Zürich, Switzerland.

Monaco

International symposium on 'Long-term effects of neuroleptics'

Monte Carlo, 18–21 March 1979

Scientific secretariat: F. Cattabeni, Milan, S. Gorini, Florence, G. Racagni, Milan, and P.F. Spano, Cagliari, c/o Fondazione Internazionale Menarini, Piazza del Carmine, 4, I-20121 Milano (Italy).

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