respect, with relatively small sections of tissue yielding cell lines which are capable of self-reproduction time and time again. Furthermore, human fibroblasts are probably used to at least the same extent as cells derived from animal tissue and the cultures thereof are likely to produce data more relevant to man than those of animal origin. It is appreciated that organ culture is more extravagant of material, but even so it is seldom that complete organs are required. In any event, the number of projects based on cell culture alone demanding investigation is such that existing research resources cannot realize the potential inherent in them within a reasonable time.

The second issue concerns the alleged necessity to use vast numbers of animals for comparative purposes when developing in-vitro techniques. Professor Gowans will doubtless have knowledge of such techniques developed without the sacrifice of the numerous additional animals which he implies is inseparable from such research. We have already obtained confirmation of the premise that this is indeed possible during the first year of operation of the Lord Dowding Fund. Grants for several such projects have been awarded and assurances received that no animals would be involved at any stage. Furthermore, a vast amount of data must have been compiled from millions of past experiments on animals, and it is realistic to assume that much of this is being utilized for assessing replacement techniques in course of development.

It is, perhaps, not surprising that Professor Gowan's observations are confined to research almost entirely concerned with in-vitro systems. This justifies our assertion that in existing research establishments there is a far too rigid demarcation, and separation into specific disciplines, of projects with little co-ordination between them. If real progress is to be made towards what we, the National Anti-Vivisection Society, would like to regard as a common objective (however euphemistic that may seem at times), the importance of co-ordinating the research of several disciplines is a factor that surely must be acknowledged and acted upon.

We appreciate the dedicated work of many scientists in the often successful attempts to develop viable alternative procedures. Likewise, we recognize that there remain fields where no such systems can at present be visualized as having viability. Nevertheless, the evidence available indicates there remain many other fields where these systems might well be developed, if only more concentrated and better co-ordinated resources were devoted to the perfecting of techniques (admittedly, often exceedingly difficult and complex) in this category.—I am, etc.,

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Effects of Entonox on Respiratory Function

SIR,—Entonox is a self-administered analgesic used in childbirth, consisting of equal volumes of nitrous oxide and oxygen.¹ It is also used in dentistry, ambulance work, and postoperative care. Previous investigations of the effects of such a mixture on pul-

monary function appear to have been restricted to measurement of the forced vital capacity capability of patients following upper abdominal surgery as an indication of pain relief (using 25% nitrous oxide, 75% oxygen).^{2,3}

We have investigated the bronchoactivity of Entonox in five adults with normal ventilatory indices by means of a whole-body plethysmograph. It is usual practice to express resistance of the airways as specific airways conductance, which is conductance divided by lung volume. After control values had been obtained the subject remained seated in the plethysmograph and inhaled from a standard B.O.C. Entonox apparatus (portable model) through the face mask provided. The exhalate was voided externally to avoid contamination of the plethysmograph. The subject took 10 deep breaths, after which the specific conductance was remeasured immediately.

The five subjects all showed a drop in specific conductance (range 19-31%; mean 26%). The subject with the largest change still showed a reduction of 13% after three minutes though the other subjects were almost back to normal within three to four minutes. The observer noted a more regular breathing pattern on the oscilloscope immediately following exposure.

Thus we feel that consideration might be given to using an alternative analgesic (for instance, methoxyflurane, which has a bronchodilator action in normal subjects⁴) for those patients presenting with airways obstruction or with a history of asthma.—We are, etc.,

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¹ *Lancet*, 1965, 1, 1229.

² Parbrook, G. D., and Kennedy, B. R., *British Medical Journal*, 1964, 2, 1303.

³ Parbrook, G. D., Rees, G. A. D., and Robertson, G. S., *British Medical Journal*, 1964, 2, 480.

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Chlorodyne Dependence

SIR,—I was interested in the cases of chlorodyne dependence reported by Dr. R. R. Parker and his colleagues (9 March, p. 427) as I was involved with a similar problem which came to notice in an unusual manner.

In November 1970 a female infant was born by rapid spontaneous delivery to an unmarried mother who had attended the antenatal clinic only once late in the pregnancy. At the age of 48 hours the infant was reported to have vomited and to have become jittery and rigid, with a shrill cry. The blood sugar level at that stage was 72 mg/100 ml. Despite intramuscular phenobarbitone the baby's general state remained the same, with repeated short-lasting jerking movements, constant tongue protrusion, and sucking and grimacing movements.

On the fourth day the baby's father told the nursing staff that the mother had been taking two bottles of Collis Browne's compound a day for many months, having

started this on the advice of a chemist for "nervous diarrhoea". Bottles of the mixture were found in the mother's locker. The baby was put on chlorpromazine and gradually settled. The chlorpromazine was discontinued after a week. The mother was seen by a psychiatrist and follow-up arrangements made for both. The baby was seen once aged 5 months, when she appeared to be developing normally, but after this the mother failed to keep appointments.

The child was next heard of in November 1973, when her general practitioner informed us that her mother had continued to take Collis Browne's compound, had failed further psychiatric attendances, had deteriorated physically and mentally, and was suspected of administering the mixture to her daughter. Although this was not proved, the constant failure to co-operate with the G.P., psychiatrist, and social services led to the little girl being admitted to a foster home under a court order.—I am, etc.,

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Vaginal Cytology in Pregnancies Associated with Congenital Fetal Defects

SIR,—Vaginal cytology during pregnancy associated with a number of abnormal maternal conditions has been described.¹ However, studies of hormone-induced cytological changes in association with abnormalities of the conceptus have mainly been concerned with hydatidiform mole.^{2,3} During the course of an investigation at St. Mary's Hospital, Manchester, into the use of vaginal cytology in pregnancy^{4,5} a series of vaginal smears were taken from 340 patients throughout their antenatal period. The smears were all taken by the same person from the lateral vaginal wall with an Ayre's spatula. The examinations carried out have been described elsewhere.⁵

In addition to maternal abnormalities in several cases there were eight babies with congenital malformations. The mothers of four of these (one with Treacher-Collins syndrome, one with imperforate anus, one with pyloric stenosis, and one with hydrocephalus) had essentially normal vaginal smears during pregnancy.

Three babies were anencephalic. The cytological features are summarized in the table. The abnormalities in the vaginal smears appeared early in pregnancy, long before the clinical diagnosis of anencephaly was made, and were probably associated with the abnormally low levels of urinary oestriol excretion described in this condition.^{6,7} In Case 2 *Trichomonas vaginalis* was present in the initial smear but otherwise there was no evidence of inflammatory disease in the vagina in this group.

The eighth patient, a 40-year-old primigravida with a clinically normal pregnancy, had persistently high karyopyknotic indices, some in the mid-trimester reaching 40%. The degree of clumping and the bacterial flora were normal, but the eosinophilic indices were, as in the anencephalic group, at the upper limit of normal. There was no vaginal inflammation. She was delivered of a mongol baby. The placenta was normal in all the parameters examined.⁴ However, she

Vaginal Cytological Findings during Pregnancy in the Mothers of Three Anencephalic Babies

Case No.	E.I.	K.I.	Vaginal Flora	Clumping	Other Features
1	Just above normal	High	Variable	Variable	Occasional parabasal cells
2	Just above normal	High	Variable	Moderate to good	Occasional parabasal cells
3	Variable; mainly normal	Variable but high (up to 40%) in 3rd trimester	Variable	Variable	Nil

E.I. = eosinophilic index
K.I. = karyopyknotic index

was having difficulties in accepting the diagnosis, and karyotyping of mother and baby was deferred. MacNaughton⁸ reviewed the evidence that mothers of such babies may in the non-pregnant state have an abnormal steroid metabolism. An increased output of dehydroepiandrosterone has been reported in young mothers of mongoloid children^{9,10} but this has been questioned by others¹¹ who discount the possibility that the differences present are due to translocations being present in only some of the mothers while others have a normal karyotype. However, the opportunity to study the hormonal status during pregnancy in such a condition does not often arise, and further studies may well indicate that some of the gene loci involved in regulating oestrogen metabolism during pregnancy reside in the affected chromosomes.

The presence of non-specific, unexplained, persistently abnormal hormonal cytological patterns in pregnancy in groups at risk may alert the clinician to the possibility of certain congenital defects being present and indicate the necessity for further investigations.

I would like to acknowledge the encouragement of Professors W. I. C. Morris and F. A. Langley and a research grant from the United Manchester Hospital.
—I am, etc.,

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Can I Have an Ambulance, Doctor?

SIR,—The article by Dr. T. C. Beer and others (9 February, p. 226) provides some very disturbing facts, not so much about the use of the ambulance service but about physiotherapy. What it reveals is that a treatment centre attached to one of the most modern hospitals in the country provides such a low level of treatment that the author's comments about the effectiveness of outpatient physiotherapy are almost bound to be correct. The last sentence is

really the important one: "Doctors ordering [physiotherapy] should remember that it may be merely a potentially socially disruptive placebo."

The facts revealed in the article showed that 75% of the patients treated were receiving no more than one hour of physiotherapy per week. I find it incredible, at a time when rehabilitation services are being reassessed and recommendations made about the implementation of the Tunbridge Committee report and when consultants in rehabilitation are being appointed, that such a very low level of treatment with a modern rehabilitation centre which treats patients on a full-time basis—that is, the patients attend for a 37-hour working week and treatment is devoted to active exercise or effective forms of physiotherapy and recovery time is reduced to an absolute minimum thereby.

I wonder if this article is in fact illustrating a norm for patient after-care because, if it is, no wonder the rehabilitation services are in such urgent need of re-appraisal and reorganization.—I am, etc.,

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Cot Deaths

SIR,—With reference to your very informative leading article (2 March, p. 341) the following factors seem *always* to be present when cot deaths occur: (1) horizontal position; (2) bed rest; (3) quiet sleep for some hours; (4) the baby is clothed and the covers are wrapped round the child; (5) a helpless and somewhat weak child; (6) an unsuspected and silent event; and (7) a greater or lesser degree of interstitial oedema in the lungs.¹

The most reasonable pathogenesis for cot deaths according to these premises is, I think, that the baby is drowned in its own body fluid. This body fluid is more abundant in newborn babies and highly mobile. Interstitial oedema in the lungs hampers oxygen transport through the respiratory membrane, especially in the hypostatic parts of the lungs. The hypoxaemia which follows produces drowsiness, insensibility, and death.

If this theory is right the fatal event should never occur when: (a) the child is out of bed; (b) the baby is crying, when the oedema would be driven out of the chest; (c) the child is naked—heavy covers in the winter season, constricting clothes, and bulky nappies under the buttocks would tend to drive more blood into the thoracic cavity; (d) the child sleeps with the upper

half of the body elevated; (e) the child has strength enough to get out of the flat, recumbent position; and (f) there has been insufficient time since washing and feeding for enough oedematous fluid to gather.

In 1969 I was telephoned by a distressed mother who had just found her 31-day-old infant motionless and apparently dead. I immediately ordered the child to be placed in a vertical position. When I saw her some 6-7 minutes later breathing was present but hardly audible. The whole body was very pale, with some traces of scum around the mouth. The head and the limbs were quite flaccid. In the next 30 minutes recovery was very rapid. The baby became normal, yawned healthily, and wanted food.² The girl has been in good health since then but somehow weaker than the two older children, who were twins.

Other potentially fatal diseases in which fluctuations in body fluid may be of decisive importance are croup³⁻⁵ (acute laryngitis) and pulmonary oedema due to heart failure. At necropsy the disastrous oedema may well be gone—as is the rule in dead bodies—into the common pool of hypostatic fluid.—I am, etc.,

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High-dose Frusemide in Renal Failure

SIR,—With reference to the paper by Professor F. Cantarovich and others (24 November 1973, p. 449) and the subsequent letter from Dr. D. Ganaval and his colleagues (9 February, p. 244) I should like to report my experience of the use of high-dose frusemide in the treatment of acute tubular necrosis and exacerbations of chronic renal failure.

In my series 10 patients were treated with frusemide, initially 1g intravenously and rising gradually to 3g over a period of seven days if no response was obtained; a control group of 10 patients were treated conventionally, without frusemide. The patients were allocated to the two groups at random and were evenly matched for age and diagnosis.

I found that in the group treated with frusemide the period of oliguria was shorter and the diuresis was greater than in the controls, so fluid retention was not a problem; there was no life-threatening hyperkalaemia and the patients were discharged earlier (mean seven days). The most practical factor was that in the control group seven patients required peritoneal dialysis, while in the group receiving high-dose frusemide only one patient required peritoneal dialysis.—I am, etc.,

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SIR,—I have read with interest the paper by Professor F. Cantarovich and others (24