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.,

BERNARD CONYERS

Campaign Director,
Lord Dowding Fund for Humane Research
London W.1

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.,

R. B. DOUGLAS

T.U.C. Centenary Institute of Occupational Health,
London School of Hygiene and Tropical Medicine,
London W.C.1

P. BROWN
K. KNIGHT
G. MILLS
G. WILSON

St. Mary's Hospital Medical School,
London W.2

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.

⁴ Douglas, R. B., and Forsey, S. M. F., *British Medical Journal*, 1973, 4, 106.

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.,

J. M. SEMMENS

Paediatric Department,
Royal West Sussex Hospital,
St. Richard's,
Chichester

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