Serum Neutralizing Antibody Tests in Tissue Culture

Coxsackie virus type	Sera with Titres of 1/4 or Greater		Significance of χ^2 test)
	Diabetics	Controls	
B1	35/102 (34%)	54/201 (27%)	Not significant
B2	47/102 (46%)	90/201 (45%)	"
B3	56/102 (55%)	107/201 (53%)	"
B4	77/102 (75%)	120/201 (59%)	$P < 0.01$
B5	46/102 (45%)	73/201 (36%)	$P < 0.05$

give a significant answer and, in view of the difficulty of obtaining a suitable control group, we doubt whether fewer than 100 patients would suffice.

It may be of interest that in our second survey 102 acute-onset diabetics have so far been compared with 201 controls by testing for the presence of serum neutralizing antibody to Coxsackie B virus at a dilution of 1/4 and a significantly greater proporion of diabetics have been found to have antibodies to Coxsackie B4 virus than controls. The provisional results are shown in table above.

In conclusion we would like to stress that, despite our further positive findings, it is still premature to draw further conclusions about their possible clinical significance. We are, however, particularly anxious that further work on the relationship between virus infection and the aetiology of diabetes should not be impeded. Both laboratory and epidemiological investigation of this problem is proceeding rapidly in the United States and large-scale studies ought similarly to be carried out in Western Europe.—We are, etc.,

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Anaesthesia in Sickle-cell States

SIR,—The plea by Drs. K. A. Oduro and J. F. Searle (9 December, p. 596) for simplicity in anaesthesia in sickle-cell states seems over-sanguine to me. Admittedly, for the trait states one would expect no more than competent anaesthesia with adequate oxygenation, maintenance of good circulation, etc., as is required for all patients. However, among the 42 patients without haemoglobin A one death associated with sickle-cell crisis occurred. It happened to one of the 21 SC patients—the group most at risk among the sickle-cell states because of their relatively high haematocrit. Surely an anaesthetic death rate of 2·5% is not that found in non-sicklers and cannot be considered acceptable?

It is known that simple procedures—5% glucose infusion, bicarbonate treatment—counteract sickling. Three reports on alkalinization are quoted, two claiming that it prevents sickle-cell crisis and one contradicting this. The latter, however, records only a failure to find acidosis in patients admitted in sickle-cell crisis—that is, after the intravascular block has become established. It cannot be claimed to have disproved that prior treatment with alkali might have prevented the crisis.

In recent years the pragmatic advice of the past has become understood on a theoretical basis. "Isotonic" glucose causes haemodilution and thereby lowers the haematocrit. It is also physiologically hypotonic, and because of the Donnan equilibrium will cause red cells to sphere and thereby lower their intracellular haemoglobin concentration. Acidosis lowers the oxygen affinity—that is, favours sickling—and the higher the pH (well within the normal range) the more does the alkaline Bohr effect raise the affinity of the haemoglobin for oxygen. Thus there is every reason why 5% glucose and alkalinization should counteract the sickling of red cells, though one would not expect that they would necessarily reverse the precipitation of deoxyhaemoglobin S in an established infarct. A failure to obtain a reversal does not permit the conclusion that these measures are useless in prevention. They are hardly so complex that they can be classed with urea treatment and exchange transfusion as unnecessary in a plea for simplicity.—I am, etc.,

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Rh Immunization in Ruptured Tubal Pregnancy

SIR,—We were interested to read the letter of Dr. R. D. Barr (4 November, p. 295), who queried the data in our paper (16 September, p. 667), and this has prompted a formal reply. We think the criticisms fall into three categories, which we will endeavour to answer.

(1) Dr. Barr quotes work done on sickle-cell disease and other haemoglobinopathies in West Africa, stating the high incidence recorded. We are aware of these findings but wish to point out that a very different state of affairs exists among South African Negroes. In our haemoglobinopathy unit no patient with an abnormal haemoglobin has been found in the indigenous populations, and haemoglobin surveys carried out in Cape Town[1] and many parts of South Africa[2] have shown that polymorphic haemoglobin variants are extremely rare. Thus we do not see high Hb F values resulting from these diseases.

(2) Dr. Barr queries the apparent excessive number of fetal cells found in the intraperitoneal cavity (44 per 150,000 maternal cells) and the maternal circulation (14 per 150,000 maternal cells) after a tubal rupture. As we did not quantitate the amount of maternal blood in the peritoneal cavity we have no way of knowing the total amount of fetal blood present. However, in the maternal circulation the maximum number of fetal cells was 14 per 150,000 maternal cells. When intraperitoneal tubal rupture occurs the total blood volume of the fetus will be present in the maternal peritoneal cavity and the blood will be absorbed until such time as laparotomy is performed and the intraperitoneal blood removed. Recently Van Iddekinge,[3] in a review of ectopic pregnancies in the South African Negro, reported that 30% have a greater than eight-week gestation period. In our study we have found that a significant fetomaternal haemorrhage (five or more fetal cells per 150,000 maternal cells in the mother's circulation) occurs in 24% of ruptured tubal pregnancies, and we believe that the higher fetal cell scores resulted from the rupture of a greater than eight-week tubal pregnancy. An 8-10-week fetus[4] has an erythrocyte count of $0{\cdot}3\text{-}0{\cdot}5 \times 10^6$/mm.^{3} In order to score 14 fetal cells per 150,000 maternal cells the total amount of fetal blood in the maternal circulation would be approximately 1·5-2·0 ml. A score of five fetal cells per 150,000 is equivalent to about 0·5 ml of fetal blood. Blood volume studies have not been performed on human embryos. However, an 8-10-week fetus is 3-8 cm in length, and weighs 1 g at eight weeks and 14 g at 12 weeks; therefore 0·5-1·5 ml of fetal blood that we found in eight of the 38 patients who were studied is not excessive. Previous studies[5,6] have shown that a score of 5-10 Rh-positive erythrocytes (0·2-0·5 ml) will result in primary immunization, and this amount was found in 24% of our patients with ruptured tubal pregnancies.

It appears that Dr. Barr is unaware that the Rh antigen has been detected in a 38-day-old fetus,[7] and therefore these cells must be considered antigenic.

(3) All the South African negro patients who were studied were Rh-positive. Rh antibody studies were therefore not performed, but this was not the point of the paper, as our intention was to demonstrate that tubal rupture results in fetomaternal haemorrhage. We are well aware of the low incidence of Rh-negativity in the South African negro,[8] and this was another reason not to look at Rh immunization in the group we studied.

I hope that these comments will answer some of the queries raised by Dr. Barr.—We are, etc.,

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Diet and Congenital Defects

SIR,—The most striking feature of the incidence of anencephaly in England and Wales over the period 1962-70 is the uniformly steady decline in the rate, with no significant variation on either side of the regression line of incidence on calendar year (−0·045 for anencephalic stillbirths). This is shown in the table below. The one relatively high rate is