Letters to the Editor

RAPID INCORPORATION OF 2,3-DIPHOSPHOGLYCERATE INTO RED BLOOD CELLS

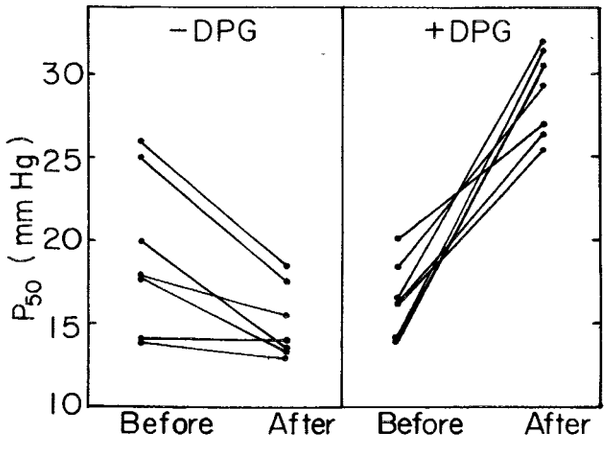
SIR,—2,3-diphosphoglyceric acid (2,3-DPG) plays a major role in regulating the oxygen transport of blood. When blood is stored the level of 2,3-DPG falls, shifting the oxygen equilibrium curve to the left and diminishing the amount of oxygen that can be released to the tissues. The incorporation of 2,3-DPG or inositol hexaphosphate into red blood cells (RBC) by biochemical or mechanical means to improve the oxygen-delivering capacity of blood is a laborious business. In 1983 Franco et al[1] reported that inositol hexaphosphate could be incorporated into RBC in the presence of DMSO (dimethylsulphoxide). We have found that 2,3-DPG can also be incorporated in the presence of DMSO, resulting in a significant right shift of the oxygen equilibrium curve.

Red cells from outdated blood in the hospital blood bank were washed with isotonic saline bis-"tris" buffer, pH 7·4. Packed RBC (1·5 ml) were mixed with 1·5 ml of 5 nmol/l 2,3-DPG (Sigma, as the pentacyclohexylammonium salt) dissolved in isotonic saline bis-tris buffer, pH 7·4. 0·19 ml of DMSO was added dropwise to bring the final concentration of DMSO to 6%. After being cooled on ice for 5 min, the mixture was rapidly dripped into 20 ml of 10 mmol/l 2,3-DPG solution at 30°C with stirring. The suspensions were incubated at room temperature for 15 min. The packed cells were collected by centrifugation (1000 *g*, 5 min) and washed three times or more with an Eppendorf microcentrifuge (10 s) until the supernatant became clear. The washed cells were resuspended in autologous plasma for the determination of oxygen equilibrium curve and measurement of 2,3-DPG. Equilibrium curves were studied, in 'Hemox' buffer, pH 7·4, at 37°C in a Hemox analyser (TCS Co, Southampton, Pennsylvania).[2] The mean±SEM P_{50} for normal blood is 27·0±0·6 mm Hg.[3] 2,3-DPG was measured spectrophotometrically[4] with the Sigma kit. The mean for normal blood is 4350±330 nmol/ml packed cells.[7] Mean corpuscular volumes (MCV) were measured with a Sysmex counter.

When RBC with very low P_{50} values were treated with DMSO without 2,3-DPG, the P_{50} value did not change whereas the P_{50} decreased for red cells with previously normal or subnormal P_{50} values (see figure). This suggests that 2,3-DPG leaked out of cells during the treatment. In contrast, treatment with DMSO in the presence of 10 mmol 2,3-DPG raised P_{50} values significantly.

To confirm that 2,3-DPG was incorporated into the cells, 2,3-DPG levels were determined enzymatically, and the results, with MCV and P_{50} values, are summarised in the table. Increases in P_{50} were accompanied by increases in 2,3-DPG, and treatment raised the MCV by 10–20%. These values were unchanged after 24 h at 4°C. The degree of haemolysis of RBC during the treatment was similar to that reported by Franco et al.[1]

To improve the oxygen delivering capacity of blood, 2,3-DPG or inositol hexaphosphate has been incorporated into red cells by several different methods. Gabrio et al[5] supplemented acid citrate dextrose with purine nucleosides and found that this maintained the levels of ATP and 2,3-DPG in red blood cells; cells remained viable



P_{50} values of blood before and after treatment with DMSO in the presence or absence of 2,3-DPG.

P_{50} VALUES, 2,3-DPG LEVELS, AND MCV OF RBC BEFORE AND AFTER TREATMENT WITH DMSO

P_{50} (mm Hg)	2,3-DPG (nmol/ml RBC)	MCV (fl)
Before treatment		
14·0	301·0	103
After DMSO alone		
13·0	281·3	136
14·5	328·2	137
After DMSO plus 2,3-DPG		
30·0 (31·0)*	7062·1 (6124·5)	119 (112)
30·5 (29·0)	7795·5 (5975·9)	118 (116)
31·5 (30·5)	7383·5 (5729·1)	108 (112)

Results after 24 h shown in parentheses.

with normal P_{50} values during storage for several weeks at 4°C. Hamasaki et al[6] reported that incubation of RBC with phosphoenolpyruvate raised ATP and 2,3-DPG levels. Nicolau and Gersonde[7] used lipid vesicles containing inositol hexaphosphate; this substance shifts the oxygen equilibrium more to the right than 2,3-DPG does. The biochemical method raises 2,3-DPG levels without damaging red cell membranes, although the incubation and removal of excess additives is very time-consuming. In contrast, the incorporation of 2,3-DPG via DMSO described here is rapid and excess DMSO and 2,3-DPG can be removed quickly by filtration. As to the mechanism of incorporation, Franco et al assumed that DMSO penetrates the red cell membrane and causes osmolarity to increase to as much as 1000 mosmol/kg. Dilution of this red cell suspension with an isotonic solution containing 2,3-DPG produces a transient osmotic gradient since DMSO leaves the cells more slowly than water enters. This osmotic stress stretches the red cell membrane, permitting the entrance of 2,3-DPG. Once DMSO has left the cells, there is no longer an osmotic imbalance and cells return to their original shape.

Although further studies, such as measurement of post-transfusion survival of treated RBC, are necessary, this method has the potential for clinical application since rapid, large-scale preparation is possible.

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SPONTANEOUS ABORTION IN PROVEN INTACT PREGNANCIES

SIR,—Does chorionic villi sampling (CVS) carry a greater risk of fetal loss than other methods of antenatal diagnosis? There is general consensus that amniocentesis in the 2nd trimester entails a risk of fetal loss of less than 0·5%,[1] whereas fetoscopy for fetal blood sampling has a risk of 0·85 to 6%.[2–4] The largest published series of women undergoing diagnostic CVS identifies 6 spontaneous

abortions in 100 procedures, one of them being chromosomally abnormal.[5] The exact time of abortion was not always mentioned. This figure corresponds with the overall abortion rate calculated from other series.

At our department ultrasonography is routinely carried out in patients reporting for their first antenatal visit.

Data on 300 patients visiting our department who all had an intact pregnancy with positive heart beat confirmed before the end of the 10th week, were analysed and the spontaneous abortion rate recorded. In the Netherlands spontaneous abortion is defined as the spontaneous expulsion of a fetus before 16 weeks of gestation. 10 patients were over age 35 at the time of expected delivery. None was over age 40. 21 patients were lost to follow-up because they did not return to the hospital and the general practitioner did not have data available. 1 patient had an induced abortion and 4 patients were excluded because they underwent amniocentesis for antenatal diagnosis. Thus, 274 case-reports were available for analysis, 236 of these were from our own population and 38 had been referred to the ultrasound department by their midwife or general practitioner.

Of the 274 patients 9 (3·3%) had a spontaneous abortion before the 16th week of pregnancy (4 of these were from the referred group). The maternal age at the time of expected delivery was 22 (2 patients), 23, 24, 27 (2 patients), 29, 34, and 35 years. No "missed abortions" were registered at follow-up beyond 16 weeks.

A prospective study of over 25 000 pregnant women showed that at the 5th, 7th, 8th and 9th week after the last menstrual period 12%, 6·7%, 5·7%, and 5% of women, respectively, will spontaneously abort before the 16th week.[6] These data do not take into account fetal viability (blighted ova and early "missed abortions" were included). Our data demonstrate that the frequency of spontaneous abortion in previously viable pregnancies, as assessed by ultrasound, is about half of the total frequency of pregnancy loss in the same period. When patients referred for ultrasound examination are excluded the abortion rate is even lower.

Our choice of study group may be criticised in that women reporting before the 10th week of pregnancy may not be representative of the whole population. Also, the sample is of women attending a university hospital pre-natal clinic and may therefore be a "high-risk" group, whereas women consulting midwives or general practitioners may be regarded as "low-risk". The loss of 21 patients to follow-up is a drawback.

The population studied differs from that reporting for CVS; in the latter group there is higher frequency of (amongst others) chromosomal abnormalities of the fetus and a higher chance of spontaneous abortion. Diagnosis of these aberrations leads to induced abortion, often before spontaneous abortion can occur. If spontaneous abortion of a chromosomally abnormal pregnancy occurs before induced abortion, analysis of the chorion sample permits differentiation of the former from spontaneous abortions attributable to CVS itself. Thus, the abortion rate of chromosomally normal pregnancies after CVS should be lower than the total abortion rate in groups where no diagnostic procedures have been carried out.

This is the first report on the frequency of spontaneous abortion in previously intact pregnancies. The very low frequency in this group must be borne in mind when informing patients of the risk of first trimester antenatal diagnosis.

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HTLV AND THE PROPAGATION OF CHRISTIANITY IN NAGASAKI

SIR,—Gallo et al[1] have suggested that human T-cell leukaemia virus (HTLV) was brought into Japan by the Portuguese in the 16th century. The virus is endemic in south-west Japan, and Nagasaki is one of the main foci. Adult T-cell leukaemia/lymphoma (ATLL) develops in HTLV carriers. HTLV is not a very communicable virus, since the endemic areas are restricted, and familial accumulation of virus carriers has been noted.[2–5] Thus, despite the passage of four centuries, it might be possible to throw light on the introduction of HTLV into the Nagasaki area by looking at the epidemiology of ATLL.

Historians record that the first visitors from Western countries to Japan were the Portuguese, who landed on Tanegashima Island, 30 km south of mainland Kyushu, in 1543. Between 1550 and 1571 several ports were opened to the Portuguese in Nagasaki Prefecture, including Hirado, Yokose, Fukunda, and Nagasaki itself. The Portuguese were missionaries as well as traders, and thousands of Japanese converted to Christianity in Nagasaki Prefecture. However, the Japanese government was anti-Christian until the middle of the 19th century. From the middle of the 17th century Japan's isolationist policy meant that Nagasaki was the only port open to western culture and only the Dutch could use it. At that time many Christians were massacred, and only those exiled to remote areas or pretending to give up their Christian beliefs survived. Most Catholics in Nagasaki Prefecture today are descendants of these people, especially in rural areas. If Gallo and colleagues' proposition is correct ATLL ought to be diagnosed more frequently in areas with a high proportion of Catholics in the population.

In Nagasaki Prefecture, 150 ATLL patients have been registered in the past ten years. The current population of Catholics was kindly supplied by the Catholic Centre, Nagasaki, and the 1980 population was taken from official statistics. As shown in the table the incidence of the ATLL was positively correlated with the density of Catholics ($r=0·92$). There were Christians in the Shimabara/Nanko area in the early 17th century, but this area was devastated in the Shimabara war against the Christians in 1637. Eradication was so complete that after the war non-Christians from all over Japan had to be brought in to cultivate the land. The low density of Catholics and the low incidence of the ATLL in this area are consistent with the historic sequence proposed and suggest the HTLV was probably already endemic at the time of the war.

The Dutch took over the trading role of the Portuguese after 1639, but a very strict isolation policy limited contact between the Japanese and the Dutch. Thus, our epidemiological findings were consistent with the concept that the virus was introduced to Nagasaki by the Portuguese in the 16th century. Since the virus is not endemic in Portugal, but is in Africa,[1,6] the real conveyer of the virus might have been Black Africans who came with the Portuguese, as suggested by Gallo et al. J. Koga's book *Prostitutes of Maruyama and Chinese/Red Hairers* (in Japanese) records the frequent contacts between the prostitutes of Nagasaki and both the Portuguese and the Indians and Black Africans associated with them.

According to K. Doi, in his 1921 *History of Syphilis* (in Japanese), syphilis was brought into Japan some thirty years before the Portuguese arrived. In those days syphilis was known as "Ryuku [Okinawa] pox" or "China pox", suggesting its origin in South-East Asia. The people of south-west Japan traded with southern China and Malaysia, where they probably had contact with the Portuguese; and syphilis is thought to have been carried to Japan indirectly via South-east Asia. However, it seems unlikely that HTLV was also brought to Japan indirectly, as with syphilis, because this would not explain the association of HTLV and Christianity. The Portuguese Christian missionaries began their work at Kagoshima, Oh'ita, Ehime, and Kohchi in the years