simpler explanation for enhancement of anti-D levels.

The published graph of variations of anti-D concentrations with time shows a rise commencing between days 5 and 7, a high peak value being achieved on day 14, with a subsequent slow fall. The pattern would fit that of a classical secondary immune response¹ due to an immunising stimulus (that is, maternal infusion of rhesus positive erythrocytes) around day 0. We suggest that the likely immunising factor was the amniocentesis and intrauterine transfusion performed on day 1.² The failure to detect fetal cells in the maternal bloodstream does not exclude the possibility of fetomaternal haemorrhage as such cells are rapidly cleared in the presence of potent anti-D.³ We therefore invite the authors to reconsider whether the interesting data that they quote on K-cell lytic potential and inhibition of lymphocyte transformation might represent facets of a well-co-ordinated immune response rather than initiating factors of that response.

The authors refer to their disappointing results of treating other cases and to their use of Rh-unselected fresh frozen plasma replacement therapy. We measured the amount of red cells present in 10 carefully prepared plasma packs prior to freezing. On average 0.06 ml of blood (at packed cell volume 0.45) was present per pack (range 0.005-0.25 ml). When a pack was frozen and thawed, 4% of the erythrocytes survived intact. If 20 donor plasmas are required for a four-litre exchange, as much as 0.05 ml of intact Rh-positive erythrocytes might be infused. Such a stimulus is capable of exciting an antibody response in a good antibody former.⁴

In an attempt to reduce the amount of donor material needed for albumin and immunoglobulin replacement during intensive plasmapheresis, we have explored, in a non-pregnant volunteer, the possibility of absorbing the patient's plasma with Rh D-positive cells to reduce the anti-D content, with subsequent use of the absorbed plasma as a replacement fluid. The absorbed plasma was centrifuged twice and filtered at 0.22 μ before reinfusion. The initial anti-D level of 27 IU/ml fell after five plasmaphereses of 1200 ml each to 14 IU/ml; this was followed by a rise within seven days to 104 IU/ml, falling within a further week to 65 IU/ml. We therefore conclude that even the stringent attempts described to remove all D-positive material from plasma were unsuccessful and that sufficient Rh D-positive cells, or stroma, or both, remained to produce a typical secondary immune response.

In conclusion, we believe that enhancement of anti-D levels can be avoided if invasive uterine procedures (amniocentesis and intra-uterine transfusions) are kept to the irreducible minimum and that only Rh-negative plasmas should be used for plasma exchange of this group of patients.

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¹ Roitt I. *Essential immunology*. Oxford: Blackwell Scientific Publications, 1977:3.

² Zipursky A, Pollack J, Chown B, Israels LG. *Lancet* 1963;ii:493-4.

³ Jandl JH, Jones AR, Castle WB. *J Clin Invest* 1957; 36:1428-59.

⁴ Jakobowicz R, Williams L, Silberman F. *Vox Sang* 1972;23:376-81.

*. We sent this letter to the authors, whose reply is printed below.—ED, *BMJ*.

SIR,—Following the submission of our recent paper (28 June, p 1569) we have evaluated the effects of plasma exchange on a further four patients with anti-D. In each of these patients a rise in anti-D was seen, commencing between days 5 and 7 and reaching a peak between days 10 and 14, despite continued plasma exchange. Anti-D levels could not be controlled by

plasma exchange in three of the five women, and they lost their babies owing to haemolytic disease of the newborn despite intrauterine transfusions. In the two cases where control of anti-D was achieved Rh(D)-negative babies were subsequently delivered. The rise in anti-D in these latter two patients could have occurred only as a result of the effects of plasma exchange. Inhibitory factors were not found in these patients' serum and we assume that Rh(D)-positive stroma was present in the fresh frozen plasma, and provided the necessary antigenic stimulus.

We therefore agree with Dr Wensley and others and with Drs D Tovey and E A E Robinson (personal communication) that anti-D levels may be boosted by Rh(D)-positive stroma. However, in the three cases where intrauterine death occurred the situation would appear to be more complex. The rise in anti-D could not be controlled by plasma exchange and it is interesting to note that plasma inhibitors of lymphocyte function were present. We would assume that Rh(D)-positive stroma (or amniocentesis) provided the necessary stimulus to anti-D production, but we would suggest that plasma exchange adversely affected the maternal control mechanisms, resulting in "escape" of antibody production and death of the Rh-positive fetuses.

It is interesting to note that the anamnestic rise in anti-D levels in the patient receiving "absorbed" plasma described by Dr Wensley and his colleagues was also associated with plasmapheresis.

The points made by Dr A C Newland and others (9 August, p 452) regarding the kinetics of anti-D production are highly relevant, and we took these into consideration when designing our plasma exchange protocol. Our aim was to undertake plasma exchange on a daily basis until the anti-D level was reduced to below 5 IU/ml (1 μ g/ml) since there is a higher risk of fetal mortality above this level.¹ The longer-term aim was to continue plasma exchanges at the appropriate interval to maintain the low level of anti-D. However, we have not been able to achieve this in practice, and would seriously doubt whether it could ever be achieved during the whole period of gestation.

Because of the active and selective transfer of IgG across the placenta we elected to exchange with fresh frozen plasma (FFP) rather than albumin (stable plasma protein solution (SPPS)) solution in an effort to maintain total IgG levels but reduce the IgG anti-D component. However, it is clear from our experience and that of others (Tovey and Robinson, personal communication) that long-term transfusion of large amounts of FFP is associated with unacceptable side effects and one is obliged to use SPPS during intensive plasma exchange.

We would agree with Dr Newland and his colleagues that in most of the reported series plasma exchange to remove anti-D could hardly be described as "intensive," and in our view the beneficial effects claimed for plasma exchange alone are as yet unproved.

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¹ Fraser ID, Tovey GH. In: Cash JD, ed. *Clinics in Haematology*, vol 5 (1). London: W B Saunders, 1976:149-63.

Pseudomonas ophthalmia neonatorum

SIR,—In their article on *pseudomonas ophthalmia neonatorum* (9 August, p 440) Dr Gaynor F Cole and others state that the commonest cause of "sticky eye" in babies is the staphylococcus.

In a study of 213 consecutive neonatal eye infections in a maternity unit in Ayrshire I found that although *Staphylococcus epidermidis* was the most frequent organism isolated, 46% of the specimens yielding this organism had no pus cells in the stained smear, suggesting normal conjunctival flora.¹ In my study I found that the organism most frequently associated with the presence of pus cells was *Streptococcus viridans*.

A previous study from Northwick Park Hospital, Middlesex, of 104 infants with conjunctivitis and 104 healthy neonates also showed that *Strep viridans* was the organism most frequently associated with conjunctivitis.²

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¹ McGill RET. *Communicable Diseases Scotland* 1979; 13(22):ix.

² Prentice MJ, Hutchison GR, Taylor-Robinson D. *Br J Ophthalmol* 1977;61:601-7.

Chlorpropamide-alcohol flushing in non-insulin-dependent diabetics

SIR,—As Drs A H Barnett and D A Pyke record further striking observations based on the chlorpropamide-alcohol flush in diabetics (26 July, p 261 and in a paper in the *Lancet*¹), some questions must be asked before diabetics too readily use it as a nostrum to predict their future health (statistically) or before economy-minded doctors apply it to the disposition of health care among diabetics.

The observations are cross-sectional in time, but used to predict the temporal course of disease. This implies a knowledge of what is "post" and what is "propter" in the associations they describe—firstly, between absence of chlorpropamide-alcohol flushing on the basis of a questionnaire and increased liability to recorded macroangiopathy among diabetics and, secondly, between indomethacin blockage of chlorpropamide-alcohol flushing and relative freedom from angiopathy among diabetics with chlorpropamide-alcohol flushing. Suppose that macroangiopathy is associated with loss of liability of skin blood flow, or with changed resting cheek temperature (which Köbberling and Weber² showed was closely associated with the increase in cheek temperature in chlorpropamide-alcohol flushing on reanalysis of the data of Leslie and Pyke,³ and which we have found an important predictor ($r=0.5$, $n=61$, $p<0.001$) of the increase in cheek temperature after intravenous injection of glucose).

Again, suppose that younger chlorpropamide-alcohol flushing-positive diabetics without clinical evidence of angiopathy and with relatively warmer cheeks have in operation a process affected by indomethacin, and so possibly involving prostaglandins, but that this process is not active when there is obvious vascular disease. We may be learning more about the effects of vascular disease on skin blood flow or the role of the skin in body temperature control than about chlorpropa-

mid-alcohol flushing (and indomethacin) as predictors of future development of vascular disease.

The loss of the differential association between chlorpropamide-alcohol flushing and macroangiopathy in diabetics diagnosed more than 15 years ago underlines the need for cautious interpretation. In table II of the paper by Drs Barnett and Pyke the patients without chlorpropamide-alcohol flushing of both age ranges are unusual in their greater liability to macroangiopathy with the shorter duration of diabetes; as so often, one wonders how patients entered a particular study and what a "total population" study would show, though realising how arduous this would be. The fixity of the chlorpropamide-alcohol flushing phenotype is an underlying assumption of the interpretation of Drs Barnett and Pyke, but its all-or-nothing character has already been questioned (by Jefferys *et al* at a recent meeting of the British Diabetic Association's medical and scientific section), while Köbberling has failed to confirm via cheek temperature measurement any increase of chlorpropamide-alcohol flushing-positivity among maturity-type onset diabetics compared to non-diabetics or even to juvenile-type onset patients.

Finally, what are the substantial reasons to postulate any "role of prostaglandins in the aetiology of both (vascular) types of complications?" from the observations that (1) maturity-onset diabetics with chlorpropamide-alcohol flushing and without angiopathy have the flush blocked by indomethacin; (2) indomethacin acts to inhibit a prostaglandin synthetase; (3) intravenous infusion of prostaglandin E is followed by facial flushing; and (4) absence of chlorpropamide-alcohol flushing is associated with increased risk of angiopathy? It is reminiscent of last year's involvement of endorphins³ in the aetiology of maturity-type onset diabetes.

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¹ Barnett AH, Spiliopoulos AJ, Pyke DA. *Lancet* 1980; ii:164-6.

² Köbberling J, Weber M. *Lancet* 1980; i:538-9.

³ Leslie RDG, Pyke DA, Stubbs WA. *Lancet* 1979; i:341-3.

Bladder cancer as a prescribed industrial disease

SIR,—The correction (16 August, p 523) to Dr F J Darby's letter (17 May, p 1229) on this topic, altering his statement that "Because the carcinogens responsible for causing urothelial tumour were withdrawn from industrial use in the early 1950s, the number of cases should show a decline over the next decade" (p 1230) to "by the mid-1960s . . ." makes the statement neither true nor any less misleading to clinicians and others who have to help certain sufferers from urothelial tumours (or their widows or widowers) to obtain such benefits as they may be entitled to under Prescribed Industrial Disease Regulations No 39.¹ In any case the correction is incomplete, for "over the next decade" must also be altered by adding some 15 years to this time because of the long latent period (averaging about 20 years) which is involved.

Perhaps it may help to restate the situation. Dr Darby's letter, which because of the address on it will be deemed to express the official view of the DHSS, was a comment on a paper by Sheena

Somerville and others entitled "Bladder cancer as a prescribed industrial disease: a guide for clinicians" (23 February, p 540), which gave help in defining under what circumstances a claim under PD 39 regulations might succeed. If it is to do so two criteria must be fulfilled—firstly, the disease must be of the right sort and, secondly, the patient must at some time after 4 July 1948 have been in insurable employment in a situation where the right chemicals were manufactured, used, or liberated, even if the employment consisted merely in maintaining or cleaning the plant used or laundering clothes used by workers in some such plants if this was done professionally in or for the plant concerned. It is not necessary, in relation to any particular case, that exposure reached or exceeded any particular level (see the appeal of G E Rushworth in 1979,² where a tribunal commissioner stated in judgment, "I agree with what was stated by a commissioner in decision R I 2/77 'that the presence of even very small quantities of a relevant substance is sufficient for the prescription of PD 39.'"). Nor is it necessary in any particular case to show that the disease was in fact causally related to the occupation in relation to which PD 39 benefit is claimed.

The "relevant substances" are set out in the regulations¹ in a form not easily understandable by those who are not chemists; but Dr Darby's phrase "the carcinogens responsible for causing urothelial tumour" will, in my opinion, inevitably be construed as being synonymous and coextensive with the PD 39 list; and because of the "authority" given to it by the letters DHSS clinicians will be tempted to ignore a patient's working history after "the mid-1960s" when considering whether to advise that PD 39 benefit should be sought.

In fact, far from a decline in the number of instances where such benefit should be sought—as opposed to any diminution of the total number of cases of occupational bladder cancer achieved by the growing control of the conditions of manufacture and use of the at present recognised carcinogenic aromatic amines since about 1950—an increase in patients entitled to benefit is to be expected. This is because it is not, on the whole, patients who have been employed in the larger manufacturing and using industries who stand in need of advice, for their employers and unions tend to take care of this, but because of new situations where exposure to the "relevant substances" occurred are coming to light as scientific knowledge increases. Unfortunately such knowledge takes a long time to reach clinicians (and apparently the DHSS) and so needy patients or their families do not get such recompense as they are in fact entitled to.

One such example is the discovery a few years ago that the induline and some nigrosine dyestuffs as manufactured in this country contained about 0.8% of 4-aminodiphenyl,³ a carcinogen at least as potent as 2-(β-naphthyl)amine in humans. Although this level of impurity just keeps it out of the "prohibited substances" class of the Carcinogenic Substances Regulations 1967⁴ it should be remembered that the 2-(β-naphthyl)amine impurity in the rubber antioxidant Nonox S, blamed as the main cause of the severe outbreak of occupational bladder cancer in the British rubber industry,⁵ was about 0.2%. Induline has uses in the dyeing industry; in the rubber and plastic industry; in making varnishes and stains; and in making typewriter ribbons, carbon paper, and inks—to quote but a few examples. How many sufferers have as yet been advised to make claims relating to the use of induline dyes or products made from them and containing some of the 4-aminodiphenyl?

If, as Dr Darby contends, the urothelial carcinogens had indeed been withdrawn by the mid-1960s, why was it necessary to pass the Carcinogenic Substances Regulations,⁴ which did not cover all the "relevant substances" listed in the PD 39 regulations (but which dealt with no other substances), and which prohibited only a few of them, in December 1967? Why, in January 1979, did the Health and Safety Commission⁶ find it necessary to send a warning letter to several British chemical suppliers pointing out the possible illegality of their offering for sale "certain substances specified as Prohibited Substances in Part II of the Regulations."¹

Could, and would, Dr Darby or some other official of the DHSS issue an authoritative list of where the "relevant substances" occur in industry or products, and of the dates, if any, when they were withdrawn? If not, clinicians and other advisers, as suggested in Sheena Somerville's paper, will still have to seek advice from sources other than the DHSS.

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¹ Department of Health and Social Security. *Prescribed industrial diseases leaflet (NI 2 1973): Disease No 39*. London: DHSS, 1973.

² Reith D. *Decisions of National Insurance Commissioner* (case No 172/1). CSI 24/79. Glasgow: 1979.

³ Parkes HG. *British Rubber Manufacturers' Association Health Research Unit Bulletin* 1977; No 20.

⁴ Carcinogenic Substances Regulations 1967: Statutory Instruments 1967, No 879. London: HMSO, 1967.

⁵ O'Connor Mr Justice. *Cassidy T and Wright C v Dunlop Rubber Co Ltd and ICI Ltd: Judgement*. London: High Court of Justice, Queen's Bench Division, 1971:25.

⁶ Health and Safety Commission. *Advice to local authorities*. LAAIC/C/3/1, appendix. London: HSE, 1979.

Sauna-induced acceleration in insulin absorption

SIR,—Dr H J Cüppers and others (26 July, p 307) have responded to my article (14 June, p 1411), in which I reported a significant rise in ¹²⁵I-insulin absorption (as measured externally by gamma counter) in diabetic patients after a Finnish sauna. In their study the authors injected healthy subjects subcutaneously with 10 U of Actrapid insulin prior to sauna (30 min at 85°C) and determined the levels of serum immunoreactive insulin following the injection. As compared with the control day, no increase in serum insulin levels was observed after the sauna. On the basis of their failure to see a rise in serum insulin levels after sauna and on previous findings in animals suggesting local degradation of insulin at the injection site,¹ Dr Cüppers and his colleagues criticise the method of external measurement of insulin disappearance from the injection site employed in my study.

If, however, one compares my results with their data more carefully, there is no discrepancy in the results: I also failed to see any significant rise in insulin absorption after the first 25 minutes of sauna. It was only after a repeat 25 minutes of sauna that insulin absorption in diabetic patients was significantly higher than on the control day at room temperature. More recently we have demonstrated that when ambient temperature was raised from 20° to 35°C diabetic patients demonstrated a 50-60% increase in insulin absorption over a four-hour period.² This rise in insulin absorption was accompanied by a 25% increment in skin temperature ($p < 0.05$) and a 2.3-fold increase in skin blood flow ($p < 0.05$). These observations, in conjunction with our results during the sauna, clearly demonstrate an enhanced insulin absorption in diabetic patients exposed to heat for 50-240 minutes.

Regarding the claim that the external measurement of insulin disappearance from the injection site "... is not suitable to assess the circulating levels of exogenous insulin following its subcutaneous injection," a variety of studies have demonstrated that (a) the radioactivity registered externally correlates with the amount of extractable insulin at the injection site³; (b) in human studies a correlation has been observed between the disappearance rate of ¹²⁵I-insulin from the injection site (as measured by external detection) and the change in blood glucose determined at rest⁴⁻⁵ or during exercise.⁶ Thus the available data indicate that the external measurement of the fall in radioactivity at the site of insulin injection reflects the disappearance rate of ¹²⁵I-labelled rapidly