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SOLUBILITY AND TRANSPORT PHENOMENA IN PERFLUOROCHEMICALS RELEVANT TO BLOOD SUBSTITUTION AND OTHER BIOMEDICAL APPLICATIONS

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Abstract - Perfluorochemical-based artificial blood substitutes are on the verge of entering medical practice. About 500 patients, mainly in Japan, have already received an emulsion of perfluorochemicals (PFCs) instead of blood, for a variety of reasons : non-availability or delayed delivery of compatible blood; blood transfusion refused on religious grounds (Jehovah's Witnesses); bloodless surgery; improvement of cerebral hypoxia, etc.. They open new prospects in therapeutics, including the treatment of heart infarct, cerebral circulatory troubles, severe burns, CO poisoning, activation of the body's defenses, or the use of PFC emulsions in angiography, organ and limb preservation, extracorporeal circulation, etc., that exploit not only the properties these emulsions have in common with blood (transport of the respiratory gases, maintenance of blood volume, osmotic and oncotic pressures, pH, etc.), but also those in which they differ from it (absence of chemical bonding of O_2, lower viscosity, smaller particle size, extraneousness to the organism, absence of sensitivity to osmolarity, mechanical resistance, etc.). The requirements to be met by the PFCs for this use : high O_2/CO_2 dissolving capacity, chemical and biological inertness, high state of purity and definition, industrial feasibility, aptitude of giving stable emulsions, fast excretion rates, are briefly commented on. The PFCs that are present prominent candidates for serving as oxygen carriers for second generation blood substitutes, including new homologous series of tailor-made ones, are critically presented. The data available on the solubility of gases in PFCs, methods of predicting them, and investigations on the mechanism of dissolution, are discussed in greater detail. The various factors that act upon the *in vivo* transport of O_2 by PFCs as compared with blood : linear *vs* sigmoïd O_2-dissolution curves with lower O_2 uptake at atmospheric pO_2 (making it necessary to have the patient breathe an O_2-enriched atmosphere); absence of chemical bonding of O_2, which permits the attainment of higher arterial pO_2, and better extraction of O_2 by the tissues (making it possible to deliver an oxygen boost to anoxic tissues); lower viscosity, which allows increased cardiac output and faster circulation, are evaluated. The factors that influence the excretion rate of the PFCs are examined. Preliminary data on the capacity of PFCs to dissolve other biologically relevant substances, as well as some of their other biomedical applications, including the culture of microorganisms and their use as radiopaque agents and as vectors for conveying drugs, are briefly presented.

I. INTRODUCTION

I.1 Foreseeable applications of perfluorochemical-based blood substitutes

The development of a substitute for natural blood capable of taking up a significant share of the transportation of the respiratory gases is desirable for many obvious reasons : temporary shortage of blood, especially for rare groups, on-site rescue of trauma victims and support during transportation to medical care centres, possibility of facing mass casualty situations as in case of disaster or warfare, extension of the benefit of transfusion to those who for religious or other reasons refuse the transfusion of natural blood, and to the less privileged developing countries which are not yet equipped for handling, storing (refrigerating) and despatching natural blood, contribution to blood conservation as in bloodless surgery, extracorporeal circulation, etc..

Among the various approaches to the problem of O_2 transport that have been considered - those based on hemoglobin or modified hemoglobin solutions (1), on modified erythrocytes (2), on synthetic metal chelates mimicking hemoglobin (3), and on perfluorochemicals (PFCs),

the last now appears to be closest to succeeding. It would avoid some of the disadvantages inherent in the transfusion of natural blood, among which are its short shelf-life, the need for typing and cross-matching (no delay in its utilisation), the risk of transmission of diseases (it is sterilizable); and it would be available at will.

The PFC-based blood substitutes will not only complement blood transfusion in preserving life when the required natural blood is not available, but also open a range of *new* prospects in therapeutics and medical research, where natural blood is inoperative or its use precluded. Their applications derive not only from the properties they have in common with blood, which include O_2 and CO_2 transportation, maintenance of blood volume, osmotic and oncotic pressures, pH, etc., but also from those properties in which they *differ* from it, viz. lower viscosity, smaller particle size, absence of binding to O_2, extraneousness to the organism, absence of sensitivity to osmolarity, resistance to mechanical stress, transparency to light, etc.. Thus it appears they can be beneficial in those critical conditions where blood circulation is hampered as in the case of heart infarct, cerebral circulatory troubles, severe burns, by allowing a better utilisation of the peripheral microcirculation, or in the treatment of various forms of anaemia, in the case of poisoning, as by carbon monoxide, or for myocardial protection during open-heart surgery with cardioplegia. They could also prove better adapted than blood for preservation of isolated organs and sectioned extremities prior to transplantation or replantation, where natural blood is too fragile to resist the mechanical effects of pumps and filters. Further therapeutic indications that are being explored include their use in echocardiography, cerebral and cardiac angiography (in combination with a contrast agent), chemo- and radiotherapy for cancer (in combination with anti-cancer drugs), etc..

I.2 Scope

In view of the considerable expansion the field is now experiencing, this review can be no means pretend to be comprehensive. It will focus more particularly on some aspects of the properties of the PFCs that are directly relevant to their biomedical applications (anesthetics (4) are excluded). It will therefore also be restricted to the rather narrow class of PFCs that, due to their characteristics, have been considered for this purpose. It is in many ways complementary to other reviews (5-7) of the authors, to which the reader is referred in particular for more extensive discussions of synthetic aspects, physiological behaviour and therapeutic indications, and more extensive bibliography. Other recent reviews of the field (8-10) and the Proceedings of the latest Symposia on Blood Substitutes (11-13) are also available.

I.3 Historical landmarks

Fig. 1. Mouse breathing a liquid PFC saturated with O_2 (by courtesy of Pr. L.C. Clark, Jr., Department of Pediatrics, University of Cincinnati).

It was only sixteen years ago that CLARK, following a pioneering inspiration, demonstrated the potential of fluorocarbons as gas carriers for biomedical applications. It was in 1966 that he and his colleague GOLLAN showed that a mouse immersed in "FX-80" (*vide infra* Section II.6) saturated with oxygen would continue to live by *breathing the liquid* (Fig. 1) (14). The same year, they reported that the isolated heart of a rat would continue to beat when perfused with the oxygen-carrying PFC (15). These experiments undoubtedly opened the way to the development of artificial blood substitutes.

Progress in the field then proceeded at a fast pace : in 1967 SLOVITER found that by using the PFCs in an *emulsified* form in a plasma substitute it was possible to accomplish simultaneously the transport of oxygen and the usual transport and regulation functions of the plasma (16). Experiments on animals then became possible and, in 1973, GEYER succeeded in keeping alive '*bloodless*' rats, i.e. rats whose blood had been exchange-perfused until their haematocrit (the red cell content of blood) had been brought down to less than 1% (17). In order to achieve this, the animals are perfused under anaesthesia with up to 30 times their normal blood volume; on awakening, the 'bloodless' rats behave in a

normal manner, and immediately resume eating, drinking, urinating, defecating, building nests, and so on. After 7 to 10 days, the plasma protein and haematocrit levels are back to normal, and the animals continue to grow and develop without apparent abnormality throughout their usual life span.

Other astounding experiments by GEYER were to show that animals could survive such exchange perfusion repeatedly five days in succession (Fig. 2) (18), or that such 'bloodless' animals survive exposure to an atmosphere containing 20% carbon monoxide, i.e. in conditions where all the O_2-transport function of all red cells, including those which are continuously produced by the organism, is totally blocked (Fig. 3) (19).

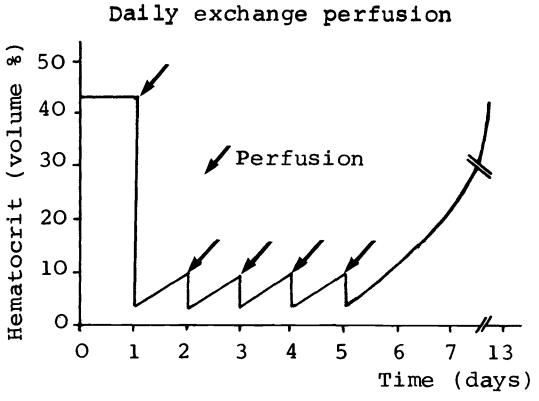

Fig. 2. Response of rat to daily repeated exchange perfusions. At the end of the series of 5 perfusions the animal remained well and returned new red cells and plasma proteins to circulation (from Ref. 18).

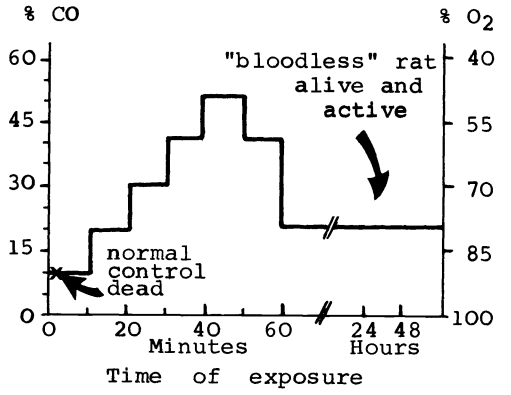

Fig. 3. Tolerance of a 'bloodless' rat to high levels of CO (up to 50% CO added at the expense of O_2). Note the early demise of the control rat (from Ref. 19)

These experiments irrefutably demonstrated that PFC emulsions can be tolerated by the organism and can deliver enough O_2 to tissues and organs for survival and, in this respect, function as blood substitutes. Unfortunately, the PFC GEYER used in most of his experiments, FC-43 (mainly tri-*F*-butylamine)[a] has a major drawback : it is retained in the organs for the rest of the animal's lifetime.

The simultaneous but independent discovery by CLARK (20) and by NAITO (21) that *F*-decalin was excreted from the body quite rapidly, with a half-residence time of only ∿ 4 days, was therefore of considerable importance. It led to the development, in 1977, by the Green Cross Corporation (Osaka, Japan), of "Fluosol-DA" (22,23), the first commercial PFC-emulsion whose use on man could be envisaged. The next decisive landmark was then in 1979, the first trial of that preparation on man - 10 volunteers among the research people of the Green Cross Corp., who received up to 500 ml of the emulsion in their veins (24).

I.4 Present state

By now about 500 patients (25,26), mainly in Japan (∿ 20 in the US), have received Fluosol-DA instead of blood, for various reasons : non-availability or delayed delivery of compatible blood; transfusion refused on religious grounds (Jehovah's Witnesses); improvement of cerebral hypoxia; anaemia; bloodless surgery - as for protection from the risk of hepatitis infection, carbon monoxide intoxication, etc..

Beneficial effects have been obtained in most cases, although "only" an average of 1.5 l (one fourth of the normal blood volume) is infused into the patient; and no untoward effects due to the PFCs have so far been reported, so that the feasibility of PFC-based artificial blood substitutes is now generally accepted, even if the achievement of entirely satisfactory and reliable emulsions still remains to work out.

Fluosol-DA (23) consists of a 20 w/v% emulsion of two PFCs, *F*-decalin and *F*-tripropylamine, in 70/30 ratio, using Pluronic F-68 and yolk phospholipids as surfactants, in a plasma substitute which contains hydroxyethyl starch as an oncotic agent (Table 1). Its viscosity (2.3 cp at 37°) is about half that of blood. The average particle size in the emulsion is 0.1μ, with no particles larger than 0.6μm.

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[a] The symbol *F* means that the compound or group that follows is *per*fluorinated.

TABLE 1. Composition (w/v %) of Fluosol-DA (20%)

F-decalin	14.0
F-tripropylamine	6.0
Pluronic F-68	2.7
yolk phospholipids	0.4
glycerol	0.8
NaCl	0.600
KCl	0.034
$MgCl_2$	0.020
$CaCl_2$	0.028
$NaHCO_3$	0.210
glucose	0.180
hydroxyethylstarch	3.0

Osmolarity : 410 mOsm

Oncotic pressure : 390 mm H_2O

The development of Fluosol-DA has given considerable impetus to the field by allowing the inception of the first experiments on humans, but it still presents numerous imperfections. Among the most serious are the insufficient definition, purity and reproducibility of the *F*-tripropylamine employed, the use of a mixture of two such oxygen carriers with widely different times of persistence in the organs, and the insufficient stability of the emulsion, which has to be stored in the frozen state, considerably limiting its practicability and commercial value. At least 40 research groups in the world, starting with those of the Green Cross Corporation, are currently actively working at formulating and testing second generation blood substitutes, which would not present these drawbacks (27).

II. PERFLUOROCHEMICALS RELEVANT TO BLOOD SUBSTITUTION

II.1 Basic requirements

The choice of PFCs to take the place of erythrocytes in their O_2/CO_2-carrying function stems primarily from their combining 1) the highest gas-dissolving capacities known for non-coordinating solvents with 2) satisfactory chemical and biological inertness. To be relevant to blood substitution, the PFCs must in addition fulfil the following requirements : 3) they must be well-defined and pure, 4) they must be industrially feasible on a large scale at reasonable cost, 5) they must give stable, fine emulsions that persist sufficiently long in the circulation, and 6) they must be excreted from the body at a reasonable rate. Items 1) and 6) will be given special attention in this review (sections III-IV); the others will only be briefly commented on here (see Ref. 6 and 7 for more details and references).

II.2 Chemical inertness and biological acceptance

Most PFCs proved to be highly inert (though the immunostimulating effects observed for some emulsions, the storage of the PFCs in *specific* organs and in tumors, and even their recently reported anti-infectious properties, can be taken as indicative of some biological activity (28)), and devoid of untoward biological effects. No evidence of their being metabolized has ever been found. When toxicity has been observed, it has generally been traceable either to the presence of some toxic impurities such as minute amounts of fluoroamines, or to some unfavourable physical characteristic such as a too-high vapour pressure or, when in the emulsified form, too-coarse particle size, or inadequate osmolarity.

An extensive array of tests has been devised to ascertain their innocuity (9). These include tissue culture, organ perfusion, various toxicity tests in animals, and lastly exchange perfusion tests in animals. In each of these tests a variety of physicochemical, physiological, haematological, morphological and histological parameters can be monitored. In organ perfusion for example, one can check both the performance of the organ and its effect on the emulsion while it is being perfused.

The most convincing facet of these tests is certainly found in the fact that animals survive, regenerate their blood and continue to live their normal life-spans after total perfusion or even repeated perfusions. The search for carcinogenic (29), teratogenic (30) and other long-term effects that have been made on some compounds has so far remained negative. It is also noteworthy that the macrophage cells which store the PFCs once they are removed from the blood stream do not seem to be affected by their extra burden, and remain perfectly healthy (28,31-36).

II.3 Purity and definition of the perfluorochemicals

It must be realized that in order to make a significant contribution to oxygen transport, the amount of substitute that has to be infused into a patient will be in the liter range - comparable to that which would be given in normal blood transfusion - which means that this patient will receive hundreds of grams of the PFC. This in turn means that *each per cent of unidentified material,* or of material whose long-term innocuity has not been fully established, *will amount to grams of that material injected into the patient;* this seems hardly acceptable, if only by health authorities.

One might therefore imagine that a high level of purity and definition of the PFCs utilised should have been a stringent prerequisite for their use in blood substitution. But this has been far from always being the case. The major part of the remarkably successful work that has been done over the past 15 years in the field has often been performed with poorly defined, notoriously complex mixtures of products.

This situation stems from the preparation procedures of the PFCs currently used, which consist in substituting fluorine for hydrogen atoms in preformed hydrocarbon derivatives. This is usually done by electrolysis in anhydrous HF (37) or under the action of high-valent metal fluorides (38). Unfortunately the large difference (*ca* 15 kcal.mol^{-1}) in C-H *vs* C-F bond energies and the release of this energy during the process provoke numerous undesired side reactions, isomerisations, rearrangements and degradations, which, in addition to incomplete fluorination, finally result in inextricable mixtures of compounds, of which some remain unidentified. At the same time it is now established that the physiological behaviour of the PFCs, including their retention characteristics and the ability of some of them to interact with cytochrome P-450, depends closely on their structure (18). Their excretion half-times, for example, can vary by a factor of 10 among isomers (section IV). It has furthermore been shown that the presence of some toxic material can be *masked* by dilution in larger quantities of atoxic material (18,39); there is no guarantee that the toxic effects will not show up at a later stage when this material is taken up by some specific cells or organs.

In the light of this, the argument that so-called "mixtures of determinate composition" could be acceptable certainly loses its pertinence, especially when their vapour phase chromatograms exhibit broad humps consisting of multiple peaks (sometimes up to 17 detectable) lumped together, making it impossible to exclude the presence of additional underlying peaks, even in the several-per-cent range, and when the relative ratios of the detected peaks vary by ± 5% from one preparation to the next (39-41).

This is why it is essential to seek more selective routes for the synthesis of PFCs - especially those routes which rely on already-perfluorinated building blocks, thus avoiding CH/CF substitutions (42) or a better control of these substitutions (43,44).

We feel that, if the use of mixtures is acceptable at all, the *individual* components of these mixtures must *all* be identified, the innocuity of *each one* verified, and its retention and other biologically determinant characteristics individually established.

II.4 Industrial feasibility

It is still difficult to evaluate the PFC-based blood substitute market, and thus that of the related PFCs, with any precision (45), but if these substitutes are to be used to an appreciable extent it is likely that this will be in the multi-hundred-tons-per-year range for the latter. The processes to be used both for their synthesis and for their purification should be able to meet such a demand at a reasonable cost. Those procedures which use elemental fluorine may in this respect be disfavoured with regard to those which use HF, on account of the considerable cost difference between these two sources of fluorine. Likewise, those materials that need numerous purification steps, including spinning band distillation and preparative vapour phase chromatography, or the refluxing of the material for several days with a 8N KOH/HNR_2 mixture - as is the case for certain electrochemically produced *F*-amines - will suffer from a serious drawback.

II.5 Emulsion stability

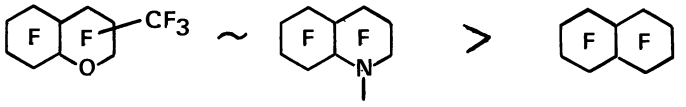
The PFCs are used in emulsified form. The surfactant most frequently used is Pluronic F-68, a polyoxyethylene polyoxypropylene block polymer, often with adjunction of yolk phospholipids, and sometimes glycerol. The emulsification procedures usually involve either sonication, i.e. use of ultrasonic vibrations, or high pressure homogenisation, as with a Manton-Gaulin homogeniser, under N_2 or CO_2 atmospheres. Oxygen, in the presence of which some degradation and the release of fluoride ions is observed, must be carefully excluded. Preliminary studies on *Micro*emulsions have resulted in the preparation of stable, very concentrated, fine emulsions, indefinitely stable within a given domain of composition and temperature, but none has to our knowledge been found biocompatible as yet (27,46,47,48).

Particle size and particle size distribution are all-important factors : not only do they determine the surface available for gaseous exchange, and in the finer emulsions their viscosity, but they also play a determinant role in the tolerability of the emulsions, for their toxicity increases rapidly when the proportion of particles larger than 0.4μ increases (quickly blocking the reticuloendothelial system), and strongly influence their intravascular persistence and the extent to which they are phagocyted by the macrophages, stored in the organs, or excreted. An average particle size of less than 0.1μ is sought.

It is also of importance that the emulsions be sufficiently stable in order to allow their transportation and long-term storage, which will largely determine the extent of their usefulness. The stability of the classic Pluronic/yolk phospholipid emulsions decreases drastically with decreasing molecular weight of the PFC. Whether the decrease in stability observed in a series of PFCs having the same number of carbon atoms in the order (26) :

aliphatic > monocyclic > bicyclic > tricyclic

should be assigned to cyclisation or simply to the decrease in molecular weight, is not clear. The same question holds, i.e. whether there is a specific effect of the heteroatom, or whether the increased stability for the series (26) :



is simply due to the increase in molecular weight. Improvement of the stability of the PFC emulsions, coupled with obtaining faster excretion of the PFCs, are the two goals that now focus most of the concern and efforts of the research groups.

II.6 Early-used perfluorocarbons, and candidates for "second-generation" blood substitutes

Table 2 groups the PFCs that have served to establish the feasibility of blood substitutes along with those which show the most promise for future developments. FX-80 is a mixture of PFCs electrochemically produced by the 3M Company, whose main constituent is *F*-butyltetrahydrofuran 1. It was used by CLARK in his historic experiments, but it provokes lung emphysema (which has been attributed to its high vapour pressure) and it was therefore abandoned. FC-43, also electrochemically produced by 3M Co., consists mainly of *F*-tributylamine 2. Widely used by GEYER, it gives very stable emulsions which are well tolerated by the animals, but it remains stored almost indefinitely in their organs, and this precluded its use on man.

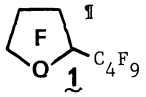
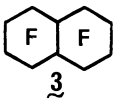
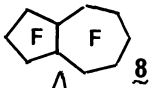
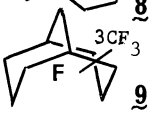
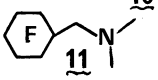
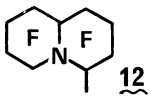
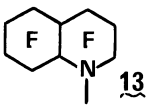
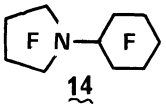
Perfluorodecalin (FDC) 3, produced by fluorination of naphthalene by CoF_3 and commercialized in pure state by ISC Chemicals Ltd, does not have this impediment. Unfortunately, all the emulsions so far prepared from it have proved rather unstable. The compromise offered by Fluosol-DA consists in improving the stability of the emulsions of *F*-decalin by the adjunction of some *F*-tripropylamine (FTPA) 4, at the cost, however, of the long-term retention of part of the PFCs, since FTPA is still detected in the organs after a year (8,32,35).

The bis-1,2-(*F*-alkyl)ethenes 5-7 have been prepared under the sponsorship of the CNRS and Produits Chimiques Ugine Kuhlmann as part of our efforts in preparing pure and unambiguously defined PFCs specially designed for biomedical uses (5,49-54). They derive from the addition of perfluoroalkyl iodides to 1-perfluoroalkylethene - both of which derive from the industrially prepared, highly pure tetrafluoroethylene - followed by dehydroiodation.

Among these, bis(*F*-butyl)ethene, 5 (code-named *F*-44E), stands out. It can be produced in 99.9% *pure* form *in any desired amount*, using straightforward preparation and purification procedures. Its chemical and biological inertness has been tested towards a range of chemical (O_2/MoO_5/HMPA, $KMnO_4$/KOH, m-$ClC_6H_4CO_3H$, Fenton's reagent, etc.) (42) and "biomimetic" (Udenfriend's reagent, chloro-α,β,γ,δ-tetraphenylporphyriniron(III)/iodosylbenzene) oxidation reagents or dehydrofluorination agents (KOH; $HNEt_2$); towards cell cultures (Namalva strain lymphoïds); by intraperitoneal injection in mice and rats, and, in emulsified form, by massive (8g/kg body weight) blood substitution experiments in rats. It displays one of the highest O_2 and CO_2 dissolving capacities known, even among PFCs, gives more stable emulsions than *F*-decalin, and has a much lower retention half-time (26,53) in the organs than *F*-tripropylamine. The more recently produced (54,55) and not yet fully evaluated (56) (*F*-i-propyl-1)(*F*-hexyl-2)ethene 6 appears to give stabler emulsions for comparable excretion rates. Bis(*F*-hexyl)ethene, which gives very stable emulsions, is retained in the body for overlong periods of time; its use in blood substitution *in vivo* is therefore precluded, but it could find applications in the perfusion of organs, preservation of sectioned limbs, cell culture technology, etc..

Phase I investigations were performed on compounds 8-11 in about 20 different formulations by several research groups in association with chemical companies (Air Products, Suntech) under NHLBI sponsorship in the US, in order to select a formulation for a second generation substitute (26,39-41,57). The four PFCs selected from about 40 synthesized and evaluated in

TABLE 2. Perfluorochemicals relevant to blood substitution

PFC	code or trade names [Molec. Weight]	Preparation definition purity	vp (mm Hg) solub. O_2 CO_2 (vol.%, 37°C)	Stability of emulsions	Retention 1/2 time (days)	Observations
 **1** C_4F_9 ¶	FX-80 FC-75 [418]	electrochem. mixtures	58	fair	several month	toxic (lung emphysema)
$N(C_4F_9)_3$ **2**	FC-43 [671]	electrochem. mixture 80-85%	1.1	excellent	>500	usable for isolated organ perfusion "Oxypherol"
Fluosol-DA (Green Cross Corp.) :						
 **3**	FDC PP5 [462]	CoF_3 >99.9%	12.5 45 126	poor	7	insufficient stability of emulsions
$N(C_3F_7)_3$ **4**	FTPA [521]	electrochem. mixture >95%	18 45 166	good	30-65*	
Bis-*F*-alkylethenes :						
$C_4F_9CH{=}CHC_4F_9$ **5**	*F*-44E [462]	>99.9%	12.6 50 247	good	8	
$i\text{-}C_3F_7CH{=}CHC_6F_{13}$ **6**	*F*-i36E [512]	>99.9%	7.6	very good		
$C_6F_{13}CH{=}CHC_6F_{13}$ **7**	*F*-66E [664]	>99.9%	2.3 41 211	excellent	>400	usable for isolated organ perfusion
NHBL contract 79-81 :						
 **8**	FBC [462]	CoF_3 67-87%	13.5	fair-poor*	5	limited industrial feasibility
 **9**	FTN [562]	CoF_3 50-55%	2.9	fair-good*	11-14 30 *	limited industrial feasibility
$(C_6F_{13})_2O$ **10**	FHE [654]	electrochem. 90-98%	11.8	excellent	60-110 >500 *	
 **11**	FMA [483]	electrochem. 80-94%	17	poor-fair*	10-11 30 *	remains slightly toxic even after 5 days reflux with KOH 8N/$HN(iBu)_2$
Green Cross Corp. "second generation" 1982 :						
 **12**	FMOQ [495]	electrochem. 95% (including isomers)	10.7 46	very good	7	require multi-step detoxification for several days. Stable 6 months at 4°C
 **13**	FHQ [495]	electrochem. 65% (major constituent 30%)	8.1 44	very good	9	
 **14**	FCHP [495]	electrochem. 87%	8.6 49	good	13	

* evaluations vary with method (see Section IV.1); sources : Ref. 10, 18, 26, 40, 41, 53, 57, 107, 120.

¶ Symbol **F** in the center of a ring structure implies that any substituent not shown at ring or side chain is fluorine.

previous projects, all consist of complex mixtures composed of a primary PFC and related isomers and other products which could not be separated by distillation, and whose compositions were reproducible at ± 5%. All contained partially fluorinated compounds which were removed by exhaustive treatment (5 days to 4 weeks) with 8N KOH and a secondary amine. They were emulsified with Pluronic F-68, yolk phospholipids and glycerol, and first evaluated for particle size and emulsion stability and, for the most satisfactory ones, for toxicity, by detailed haematologic, biochemical and histological analysis at 50% haemodilution in rats in air. The organ half-life and expiration rate were then determined. The formulations which have been retained for Phase II evaluation are those based on *F*-trimethylbicyclo[3.3.1]nonane and on a 6:4 mixture of this with *F*-N,N-dimethylcyclohexylmethylamine. So far their superiority over Fluosol-DA is, however, not evident.

The 3 components 12-14 retained by the Green Cross Corp. for Phase II evaluation, result from an impressive, thorough screening of 54 PFCs (26). Besides absence of toxicity, better emulsion stability than that of Fluosol-DA, combined with half retention times of less than two weeks, were the main criteria of selection. As for those selected in the NHLBI project, most of these materials suffer from low purity, with one main constituent usually present in the range of 30-95%, and their large-scale production may be seriously hampered by the constraining and lengthy detoxification processes they require.

III. THE SOLUBILITY OF GASES IN PERFLUOROCHEMICALS

III.1 Solubility data and methods

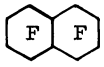
The solubilities of O_2, CO_2 and other gases in a range of PFCs and, for comparison, in a few other chemicals, are illustrated in Table 3. The sources of these data are widely scattered; when several values are available for a given PFC and gas, considerable discrepancies can be noted. These may have various origins, including the diversity of methods employed, some of which may not be adapted to the problem; the lack of standardisation of the experimental procedures and conditions; and the uncertainty about the exact constitution of the PFC samples. The methods most frequently used on pure PFCs are manometric (58) or volumetric (as with Van Slyke's apparatus) (59), chromatographic (using 5Å molecular sieves for O_2 and N_2, and Poropak P columns for CO_2) (60-62), and NMR in the case of O_2 (63). Gas solubilities in emulsions were obtained by chromatographic (64) and polarographic (65) methods, as well as by redox titration after oxidation of ferrous thiocyanate by the oxygen contained in the emulsion (66), or enzymatic determinations using glucose oxidase in the presence of excess glucose (67). Ref. 61 lists the VPC-determined O_2 solubilities and other useful data for a range of 25 highly perfluorinated chemicals, and attempts to correlate them. A detailed experimental procedure is given.

Generally speaking, the solubilities of gases in PFCs are higher than in water by a factor of 20 or more if expressed in vol.%, or of 200 if expressed in molar fractions, but differ only by a factor of less than 3 from those in related hydrocarbons : it should be clear that it is water which has an exceptionally *low* gas-dissolving ability. Solubility is greater in aliphatic than in aromatic PFCs, with the exception of CO_2, and decreases as fluorine atoms are replaced by hydrogen atoms. *Differences* in O_2-dissolving capacity in aliphatic PFCs having 9-11 carbon atoms are far from negligible; they range from 40 to 50 vol.%, and should certainly be taken into account in the choice of a gas-carrier, in order to help reduce the O_2 content of the atmosphere which the patients have to breathe (Section III.4). The gas-dissolving capacity decreases in the order : $CO_2 \gg O_2 > CO > N_2 > H_2 > He$, apparently following the decrease in molecular volume of the solute. The solubilities of various gases in a given solvent may either increase (as in the case of H_2 or He), remain almost constant (N_2, Ar), or decrease (CO_2, gaseous hydrocarbons) with increasing temperature, but they tend to reach the same value at the critical temperature of the solvent (68).

The solubility varies linearly with the gas's partial pressure, according to Henry's law. Some authors (59,64) noticed a decrease in O_2 solubility (in vol.%) in FC-80 or FC-43 when O_2 was progressively diluted in N_2 or He (but the validity of using Van Slyke's method in one case (59) has been questioned (67), because the O_2 extracted from the sample at reduced pressure may redissolve in the PFC during the completion of the measurement). Other reports (60, 61) indicate an opposite trend. The gas-dissolving capacities of the PFC-*emulsions* are close to those predicted by adding the values obtained for the two phases separately (65,66).

The enthalpies and entropies of solution are small, about -4kJ.mole^{-1} and -50 J.mole^{-1}.K^{-1} respectively, but increase in magnitude with increased molecular complexity (69).

TABLE 3 : Gas-solubility data for selected perfluorocompounds and related chemicals.*

Compound	O_2 Meas.	O_2 Calc (74)	CO_2	N_2	CO	H_2	He
$n\text{-}C_7F_{16}$	54.8[a]		207.0[b]	38.6[c]	38.6[a]	14.1[d]	8.81[b]
$n\text{-}C_7H_{16}$	15.2[a]; 16.7[e]				26.3[a]	10.4[d]	3.8[f]
$n\text{-}C_8F_{18}$	52.1[g]						
$n\text{-}C_8H_{18}$	28.8[e]			18.0[e]		9.4[d]	3.3[f]
$n\text{-}C_9F_{20}$	49.6[g]						
$n\text{-}C_9H_{20}$	26.5[e]			16.1[e]		8.7[e]	2.96[f]
	48.8[g]; 46.8[h]		426[h]	34.8[h]	41.1[h]		4.1[h]
	20.6[i]		245,5[j]	11.3[i]	16.9[i]	6.5[d]	1.95[f]
	57.2[g]	58.1[k]		32.8[c]			8.6[l]
	30.5[m]		178.4[m]	18,0[m]	23.8[m]		2.86[l]
CCl_4	27.8[i]		243.7[i]	14.9[i]	20.3[i]	7.73[d]	
Acetone	25.6[i]		565.0[n]	16.4[i]	23.5[i]	9.14[i]	3.3[o]
Ethanol	22.5[p]		244.7[n]	13.8[p]	18.6[q]	7.95[r]	2.96[o]
H_2O	3.1[s]		82.8[s]	1.59[s]	2.33[s]	1.91[s]	0.95[s]
$C_8F_{17}CH{=}CH_2$	38.6[t]	49.0[k]					
$C_8F_{17}C_2H_5$	38.9[t]	49.1[k]					
$C_4F_9CH{=}CHC_4F_9$	*50.1*[u]	49.9[k]	*247*[u]				
$C_6F_{13}CH{=}CHC_4F_9$	*44.6*[u]	44.4[k]	*240.6*[u]				
$C_6F_{13}CH{=}CHC_6F_{13}$	36.6[t]; *40.8*[u]	42.1[k]	*211.4*[u]				
$C_4F_9CH{=}CHC_2F_5$	*52.8*[v]	51.2[k]	*268*[v]				
	40.3[g]	47.1[w] 42.7[k]	179.7[w]	29.5[w]	32.4[w]		7,5[w]
	52.2[g]; 46.9[x] 50.2[aa]; 65[bb]	48.8[y] *48.5*[z]	192[y] *160*[z]	33.4[y] *36.8*[ee]			
(FC-80)	*48*[z] ; *39*[cc]		*135*[dd]				
(FC-75)	*45.5*[ff]; *71.4*[ee]		*235*[cc] *179.3*[ff]				
$N(C_3F_7)_3$	*45.3*[v]	49.6[k]	*166*[v]				
$N(C_4F_9)_3$	33.2[b]; 38.4[g]	44.8[k]	127[b]	22.3[b]			
(FC-43)	37.5[x]; 38.9[y]		152[y]	28.4[y]			
(FC-47)	37[bb] *40.3*[y]; *37*[z]		*142*[y] *140*[z]	26.9[dd] *22.4*[ee]			
	32.2[ee]; *36.2*[ff]		*110*[ee]	*28.6*[y]			

* see also TABLE 2 ; *data in italics measured at 37°C*, otherwise 25°C; values from the literature that were calculated or estimated are not reported.

TABLE 3 continued :

a) see Ref. 58; b) Y. Kobatake and J.H. Hildebrand, *J. Phys. Chem. 65,* 331 (1961); c) J.C. Gjaldbaek and J.H. Hildebrand, *J. Am. Chem. Soc. 71,* 3147 (1949); d) M.W. Cook, D.N. Hanson and B.J. Alder, *J. Chem. Phys. 26,* 748 (1957); e) E.S. Thomsen and J.C. Gjaldbaek, *Act. Chem. Scand. 17,* 127 (1963); f) H.L. Clever, R. Battino, J.H. Saylor and P.M. Gross, *J. Phys. Chem. 61,* 1078 (1957); g) see Ref. 61; h) F.D. Evans and R. Battino, *J. Chem. Thermodyn. 3,*, 753 (1971); i) J. Horiuti, *Sci. Pap. Inst. Phys. Chem. Res. Tokyo 17,* 125 (1931); j) J.C. Gjaldbaek, *Act. Chem. Scand. 7,* 534 (1953); k) D.D. Lawson, J. Moacanin, K.V. Scherer, T.F. Terranova and J.D. Ingman, *J. Fluorine Chem. 12,* 221 (1978); l) H.L. Clever, J.H. Saylor and P.M. Gross, *J. Phys. Chem. 62,* 89 (1958); m) L.R. Field, E. Wilhelm and R. Battino, *J. Chem. Thermodyn. 6,* 237 (1974); n) W. Kunerth, *Phys. Rev. 19,* 512 (1922), o) A. Lannung, *J. Am. Chem. Soc. 52,* 68 (1930); p) C.B. Kretschmer, J. Nowakowska and R. Wiebe, *Ind. Eng. Chem. 38,* 506 (1946); q) J.C. Gjaldbaek, *Kgl. Dan. Widensk, Selsk. Mat. Fys. Medd. 13,* 24 (1948); r) E.B. Maxted and C.H. Moon, *Trans Faraday Soc. 32,* 769 (1936); s) E. Wilhelm, R. Battino and R.J. Wilcock, *Chem. Rev. 77,* 219 (1977); t) see Ref. 63b; u) see Ref. 53; v) K. Yokoyama, Personal communication; w) D.W. Cottrell, *Proc. Symp. Research on Perfluorochemicals in Medicine and Biology* (April 1977, Stockholm; V. Novakovska, R. Plantin Ed.; Karalinska Institute Research Centre, Huddinge University Hospital, S-14186 Huddinge, Sweden) p. 32; x) see Ref. 59; y) see Ref. 60; z) P.S. Malchesky and Y. Nose, *Fluoride 6,* 84 (1970); aa) see Ref. 67; bb) P.S. Malchesky and Y. Nose, *Advances in Cardiology 6,* 79 (1971); cc) W.H. Mears and R.L. Beavers, *Fed. Proc. 29,* 1819 (1970); dd) see Ref. 62; ee) R.P. Geyer, *New Engl. J. Med. 289,* 1077 (1973); ff) see Ref. 64.

III.2 Prediction of oxygen-dissolving capacities and other thermodynamic properties of perfluorochemicals

Most rationalisations and predictions of the solution behaviour of fluids still derive from HILDEBRAND's early work, and are based on his solubility parameter δ, which is meant to describe their internal pressure, and is defined as (70) :

$$\delta = \left(\frac{\Delta E_v}{V}\right)^{1/2} \qquad \text{where} \quad \Delta E_v = \text{energy of vaporisation (cal.mole}^{-1}\text{)}, \quad V = \text{molar volume (cm}^3\text{.mole}^{-1}\text{)}$$

In order to be mutually soluble, two fluids must have comparable values of δ. Those of PFCs are close to 6, as compared to hydrocarbons : δ = 7-9, or water: δ = 23.4, while for O_2, δ = 5.70.

Semi-empirical approaches to gas solubilities derive from the scaled particle theory, which expresses the free energy of solution as the sum of the free energy for formation of cavities in the solvent to host the solute molecules, and of the free energy of interaction between solute and solvent molecules (71). Several models have been devised to estimate these free energies and have been critically assessed in a recent review (69). Correlations are therefore expected to be observed between O_2 solubility and surface tension, compressibility (72) or viscosity (61) of the solvent, since these are macroscopic manifestations of the degree of internal cohesion of the solvent molecule.

A group additivity system developed by FEDORS for hydrocarbons (73) has been adapted to PFCs by LAWSON *et al*, and makes it possible to predict, from the structural formulas alone, the energy of vaporisation (ΔE_v^{298}) and the molar volume (V), and from these two parameters, the vapour pressure (v.p.) and the solubility of oxygen, for an extensive range of PFCs (74).

Using a set of PFCs whose heat of vaporisation (ΔH_v^{298}) and boiling point (T_b°K) were known, LAWSON *et al* adapted the empirical relationship proposed by HILDEBRAND and SCOTT (70) to PFCs. These relationships between the two parameters then become :

$$\Delta H_v^{298} = 0.0724\ T_b^2 - 17.17\ T_b + 5309 \qquad (25°,\ 1\ \text{atm.})$$

$$T_b\,°K = \frac{17.17 + [294.9 - 0.2896\ (5309 - \Delta H_v^{298})]^{1/2}}{0.1448}$$

The authors calculated ΔH_v^{298} for a large number of PFCs from their boiling points, and subsequently derived contributions to the energy of vaporisation for each individual structural component of the molecule. The molar volumes (V) calculated from the densities were similarly apportioned. By summing these individual group contributions, useful estimations of the ΔE_v^{298} and V can be obtained for any given structure, from which ΔH_v^{298}, b.p. and densities can also be predicted.

The equation that related the vapour pressure at the temperature T(°K) of a non-polar liquid to its heat of vaporisation :

$$p = T\left(\frac{\Delta H_v}{\alpha RT}\right)^{1/(\beta-1)}$$

was also fitted with new constants specific to PFCs (α = 12.2497; β = 0.8846). A simpler expression has however been recommended by PATRICK as giving better agreement with experimental results (69) :

$$\ln[P/\text{mm Hg}] = 6.6333 + [10.80 + 3.40(1-\theta)][1-1/\theta]$$

where $\theta = T/T_b$ (T_b = boiling point in ° Kelvin).

Fig. 4 compares the two approaches with experimental data for a homogenous series of bis(*F*-alkyl)ethenes (75).

The gas solubilities were then predicted on the basis of HILDEBRAND's regular solution theory with a FLORY-HUGGINS type correction to account for the difference in size between the solvent and gas molecules (76). A reasonable correlation was found between the partial molar volume of O_2 in solution and the logarithm of entropy of vaporisation of the solvent, though separate equations were obtained for open-chain and cyclic PFCs. Some data calculated in this way are reported in Table 3.

III.3 Mechanism of dissolution

No evidence of specific interactions, coordination or charge-transfer complexes, has been found between the PFCs and the gas molecules they dissolve. Their behaviour in this respect appears typical of non-polar, non-associated liquids whose structure and gas-dissolving capacity are, according to CHANDLER, essentially determined by their shape (77).

In the case of O_2 the mechanism of dissolution has been thoroughly investigated by DELPUECH *et al*, using the perturbation induced in the nuclear relaxation of the ^{13}C nuclei of the solvent molecules by the presence of the paramagnetic molecular oxygen in solution (63). The data collected for 18 solvents, including essentially linear aliphatic, cyclic and aromatic PFCs established a correlation between the solubility of oxygen and a relaxation coefficient q_x, which expresses the extent of the perturbation of the relaxation rate T_1^{-1} per mole fraction of dissolved O_2 (figure 5). The higher the solubility of O_2, the lower the relaxation coefficient : this is consistent with the dissolving capacity's being related to the ease of formation of cavities capable of accommodating the small solute molecules within the solvent, rather than to any specific interaction. The increase in relaxation coefficient from aliphatic to cyclic to aromatic structures, and the related decrease in oxygen solubilities, are thus rationalized on the basis of cavities of decreasing size. The linear aliphatic structures probably more easily allow the formation of large channels within the liquid, while the planar structures would result in more closely interlocked layers, less favorable to the inclusion of solute molecules. A change in slope shown in figure 5 has been assigned to a change in the mechanism of diffusion of the oxygen molecules from one cavity to another in the solvent, from discontinuous to continuous. The case of hexafluorobenzene appears to be singular, and the existence of specific interactions between the gas and PFC molecules has been suggested (69,78).

The higher oxygen dissolving capacity of *trans*-bis(*F*-alkyl)ethenes in comparison with their saturated analogues suggests that it should be traced to the presence of the double bond. But the fact that the related compounds having the double bond in terminal rather than in central position did not exhibit any higher dissolving capacity than their saturated analogues shows again that it cannot be accounted for by the usual electronic properties of the double bond through the formation of some charge-transfer complex. It has therefore been assigned (63b) to the "notch" introduced by the double bond into the molecular structure, which is thought to facilitate the formation of cavities capable of hosting the solute molecules, as well as their diffusion outside these cavities.

The compressibilities of a variety of PFCs have also been measured, and their internal pressure calculated (72). The compressibilities are much higher and the internal pressures lower than those of their hydrocarbon analogues, reflecting lower intermolecular interactions. Good correlation was found between the isothermal compressibility factor $\beta_T = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T$ and the solubility of O_2, N_2, He and Ar, but not CO_2. These results further support the view that the solubility of gases in the perfluorinated liquids is related to the existence of cavities that can accommodate them.

A recent *ab initio* investigation of the interactions of simple PFCs with O_2, N_2 and CO_2 indicates, rather confusingly, the formation of "stable complexes" with distinctively larger interactions than for hydrocarbons, and for which specific geometries are predicted, to finally point to simple physical dissolution in view of the weakness of the interaction energies (79).

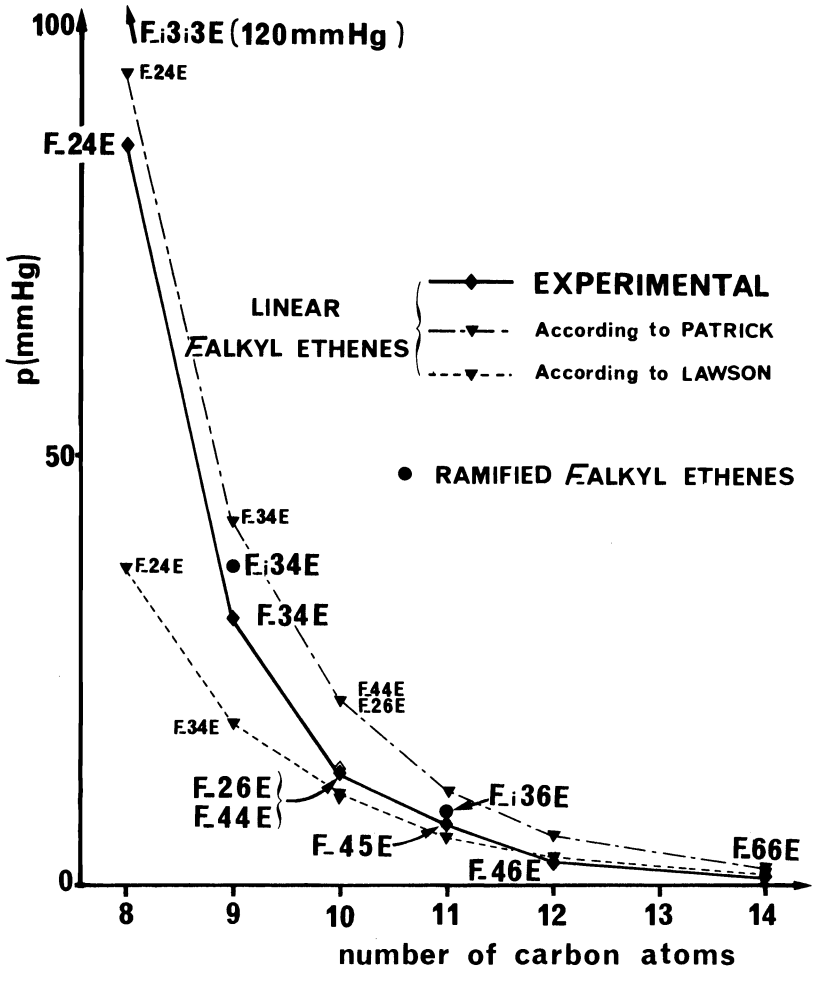


Fig.4 : Experimental vapour pressures of a homologous series of linear bis(*F*-alkyl)ethenes (75) compared to data calculated according to PATRICK (69) and according to LAWSON (73); a few data concerning ramified bis(*F*-alkyl)ethenes (55) are also given.

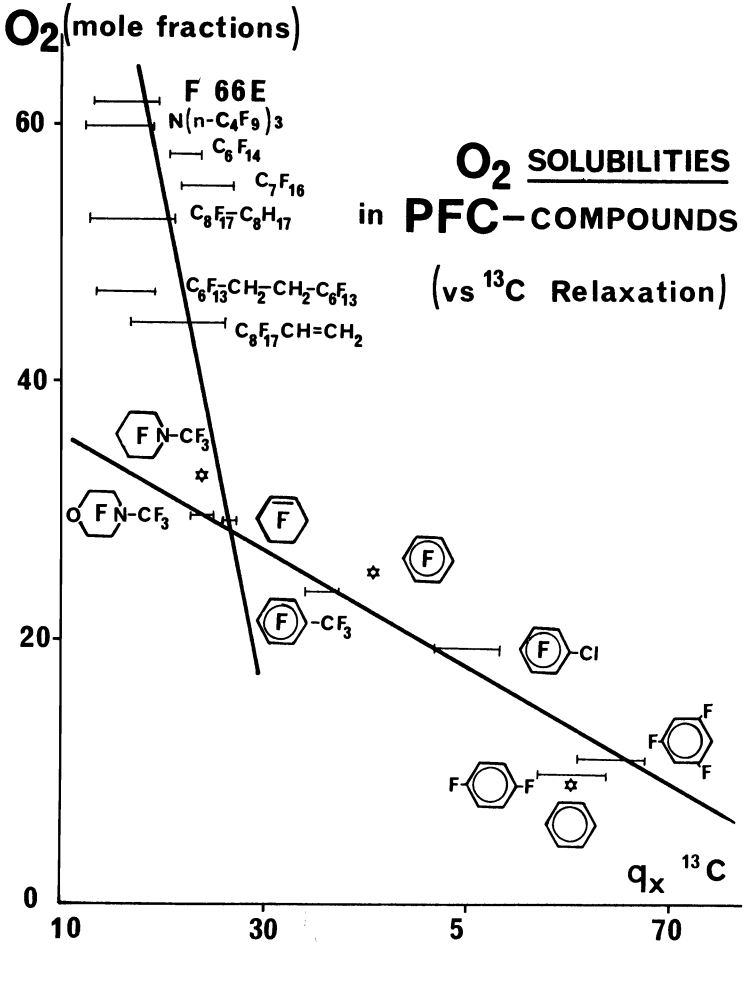


Fig.5 : Oxygen-dissolving capacity of a range of PFCs *vs* a relaxation coefficient q_x for the ^{13}C nuclei of the solvent (see text); after DELPUECH *et al* (63).

III.4 Availability of O_2 from PFC-emulsions *vs* blood

The direct comparison of the solubilities of O_2 in blood and in the PFCs has little meaning, since the mechanisms by which the gas is carried, and therefore its *availability*, are completely different. Figure 6, in which the oxygen contents are plotted against pO_2 for normal blood, diluted blood, plasma and Fluosol-DA 20% and 35%, illustrates this point.

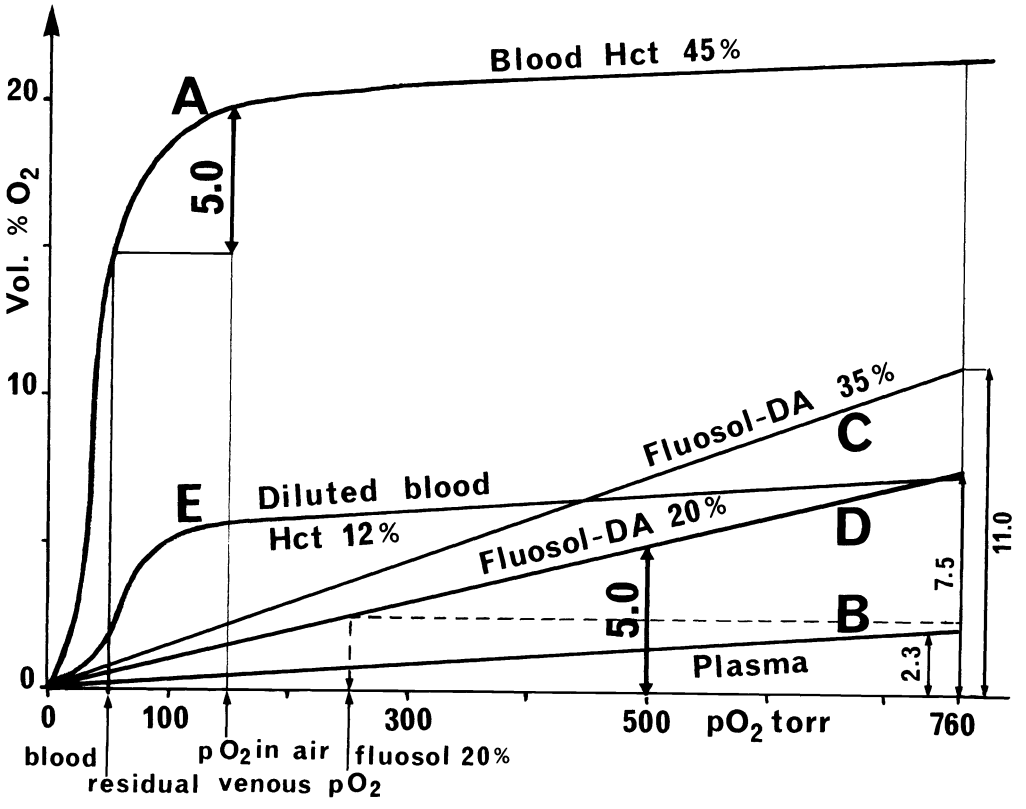


Fig. 6. Comparison of O_2 content and availability in normal blood (A), diluted blood (E), plasma (B), Fluosol-DA 20% (C) and 35% (D) as a function of oxygen partial pressure. Hct = haematocrit.

In blood, oxygen is coordinated to the haemoglobin's four iron atoms, and the O_2 uptake curve (A) has a characteristic sigmoid shape, and rapidly levels off to a straight line almost parallel to that found for the dissolution of O_2 in plasma (curve B). By contrast, the volume of O_2 dissolved in the PFC-emulsions increases linearly according to Henry's law (curves C and D).

Under biological conditions, the O_2 content of the arterial blood is *ca* 20 vol.%; it drops to *ca* 15 vol.% for venous blood after uptake of the oxygen by the tissues. One can see from Fig. 6 that natural blood (curve A) can easily release these 5 vol.% of oxygen when the animal breathes air. This corresponds, on the straight vertical part of the sigmoid curve, to a drop in pO_2 from 150 torr (the pO_2 in air, at which pressure haemoglobin is already 95-98% saturated) to *ca* 50 torr. Since the pO_2 needed in tissues is only~40 torr, this means that the O_2-delivering capacity of blood has been only partially used. *Ca* 4 vol.% of O_2 can still be provided by diluted blood (curve E) having its haematocrit reduced to $\sim$ 12 vol.%, i.e. the limit for survival. When the same variation in pressure is applied to the 20 w/v% PFC-emulsion (curve C), it can release only *ca* 1 vol.% of O_2. In order to have a dissolving capability similar to that of blood, the emulsion has therefore to function under a pO_2 of *ca* 500 torr. When pO_2 attains 760 torr, the solubility of O_2 becomes comparable in diluted blood and in Fluosol-DA 20%, but the residual pO_2 in the venous system after 5% O_2-delivery remains much higher with the PFC-emulsion (dashed line) than with diluted blood; and this is of considerable importance *in vivo* (Section III.5).

Another essential dissimilarity between blood - or other O_2-binding chelate-based systems - and PFCs lies in the differences in strength of their interaction with the gas molecule, and in the differences in oxygenation/deoxygenation *rate*. *In vitro* kinetic studies of O_2 and CO_2

transport into or from the particles in the emulsions were performed using UV stopped-flow (80,81) and polarographic (82) techniques. At 25°C the half-time for the oxygenation/deoxygenation process was found to be only 2.10^{-3}s for a 10% FC-43 emulsion, compared to 7.10^{-3}s for haemoglobin solutions; the exchange of CO_2 proceeds even faster (81). Polarographic studies seem to indicate that the equilibrium distribution of O_2 between the inside and outside of the PFC-particles is rapidly attained and that the release of O_2 from saturated emulsions is rate-determined by oxygen diffusion inside the particles, itself determined by the inner viscosity of the PFC (82). The surfactant layer around the particles appears not to play any appreciable role as permeability barrier to O_2 transportation. The range of electroactivity of the emulsions having been found analogous to that of water for a variety of electrodes, it was concluded that the usual electrochemical methods could be applied to these media to obtain data on the characteristics of the whole emulsion and not only on the aqueous phase (83).

III.5 *In vivo* oxygen *delivery* by perfluorochemicals *vs* blood

Under atmospheric pO_2, PFCs are much less efficient O_2-*dissolving* agents than blood. But it is not the O_2-dissolving capacity, but the O_2-*delivery* that is important physiologically. Fortunately the ability of PFC-emulsions *vs* blood to deliver oxygen is influenced by several favorable factors, which again stem from the *differences* between the two O_2-carrying systems and include : 1) their lower viscosity, which permits higher cardiac output, hence higher O_2-flux (84), as well as better recuperation of hampered microcirculation; 2) the possibility of attaining considerably *higher arterial* pO_2 (PaO_2), hence higher *diffusion rates* of O_2 from the capillaries to the tissues; 3) the lower affinity of PFCs compared to haemoglobin for the O_2 molecule, which, in conjunction with higher diffusion rates, permits its *better extraction* by the tissues; 4) smaller particle size, which allows better micro-flux and leads to specific indications. In addition, one should also mention the beneficial plasma expander effects on haemodynamics (25,85-86). A few selected experiments will illustrate these various points; additional examples may be found in Ref. 11-13.

Several of the above factors combine to permit considerably higher O_2-delivery/O_2-dissolution *ratios* with PFC-emulsions than with blood, and hence significant contributions of the PFCs to O_2-delivery even for relatively low doses of emulsion, provided the O_2 content of the atmosphere inspired by the patient (FiO_2) is high. Thus MITSUNO estimated that, in patients having received $\sim$1000 ml of Fluosol-DA 20%, an average of 80% of the O_2 carried by the PFC *vs* only 17% of that carried by haemoglobin was consumed. In other words, the PFC supplied 12-13% of the oxygen consumed, while it transported only 2.5% (25). The more severely anaemic the patients, the more vital this factor becomes (87-90). No adverse effects due to this increased FiO_2 - patients have usually been given 50-60% O_2 - have been reported. As for bloodless rats, they withstood 100% pure O_2 for two days and more without apparent difficulty (19).

LUTZ found that it is possible to sustain a normal arterial O_2 content of 6.1 ml/dl in massively perfused rats (10 g PFC/kg body weight) with a haemoglobin content of only 1.9 g/dl (normal 15 g/dl) under pure O_2 (91). He also observed that the basal O_2-consumption of Fluosol-DA-perfused liver was significantly higher than with blood - in spite of the fact that the O_2 supply was definitely lower - due to excessively high O_2-*extraction* at equal flow rate. For example, in the metabolic process following the administration of aminoacids to the liver, the O_2-extraction from PFCs was estimated at 95 ± 1.1%, compared to 53 ± 5% for diluted blood (at Hct 12-15%). By contrast, merely increasing the O_2-supply does not enhance O_2-consumption significantly, either with normal or with diluted blood. This remarkable O_2-extraction was assigned to the several-hundredfold increase in area and fast diffusion of O_2 with respect to red cells (92).

The O_2 *diffusion rate* from capillaries to tissues increases indeed with the pO_2 gradient. Haemoglobin being already saturated under 100 torr, and the oxygen content of blood increasing only very slowly with higher pO_2, it is useless to increase FiO_2. Thus, under normal physiologic conditions, the O_2 gradient for diffusion from the capillaries to the tissues is of *ca* 60 torr, and decreases as oxygen leaves the blood. With PFC-emulsions the diffusion gradient can easily be augmented to 400 torr, to provoke initial diffusion rates six times faster (93). Thus even small volumes of O_2 carried under these conditions may prove highly beneficial, especially in microvascular occlusive disease such as arteriosclerotic coronary or cerebral vessel diseases, or extended burns, by increasing the diffusion rates into poorly vascularized tissues.

Another all-important aspect to be taken into account when comparing O_2-transport to tissues by blood and by PFC-emulsions arises therefore from the distinct (sigmoid *vs* linear) O_2-dissolution *vs* pressure relationships : even though the O_2-*content* remaining in the venous vessels after extraction by the tissues is lower in PFCs, the latter, under high FiO_2, still exerts a higher O_2-tension than blood or diluted blood, whose venous tension does not depend significantly upon pressure and remains around 50-70 torr. This makes it possible to hold organs under high pO_2, when desired, and provides new ways of controlling tissue pO_2 by

setting FiO_2, hence pO_2 of arterial blood to the appropriate value. Thus for example after equal O_2 consumption, the PFC emulsion still exerts a higher O_2 tension - 250 torr - than blood itself (see Fig. 6). Similar remarkable increases in pO_2 from an average of 50 torr in controls to several hundreds under high FiO_2 were observed in liver, pancreas, kidney and skeletal muscle in dogs perfused with Fluosol-DA 35% (94).

The lower *viscosity* of the commonly-used PFC-emulsions, besides allowing a higher cardiac output and O_2-flux, plays a decisive role when microcirculation disturbances are involved, as in haemorrhagic shock and microvascular occlusive diseases (8,95-99). When for example haemorrhagic shock was simulated in dogs by bleeding them to a mean arterial blood pressure of only 40 torr, and isovolemic amounts of hydroxyethyl starch (an oncotic agent) solution, blood, and Fluosol-DA were infused into the animals while breathing pure oxygen, the survival rate, after 48 hours, was 80% in the Fluosol-DA-20%-treated animals, compared to only 20% in the hydroxyethyl starch- and blood-treated animals; less success (50% survival) was obtained with the more viscous Fluosol-DA 35% (100). By contrast, dogs breathing pure oxygen can survive extreme haemodilution, to 3.8-4.8 g haemoglobin per dl, with either hydroxyethyl starch solution or Fluosol-DA 20% (though only those treated with Fluosol-DA survive when CO is added to the inspired gas). Thus haemorrhagic shock, where the microcirculation disturbances are the main problem, is a situation that must be considered distinct from simple isovolemic haemodilution, and the survival rates in the former case do not depend only on the O_2-concentration. Physically dissolved O_2 under high pO_2 and within a solution of low viscosity was considered the only possibility of carrying oxygen into the capillaries in this case (100-101).

The effect of Fluosol-DA, compared to a hydroxyethyl starch (HES) solution, on tissue oxygenation during normovolemic haemodilution in dogs has been investigated by OKADA *et al* (Fig. 7) (102). Two groups of dogs were infused, one with Fluosol-DA and the other with the HES solution, so as to reach haematocrits (Hct) of 20% and 10% successively, while the same volume

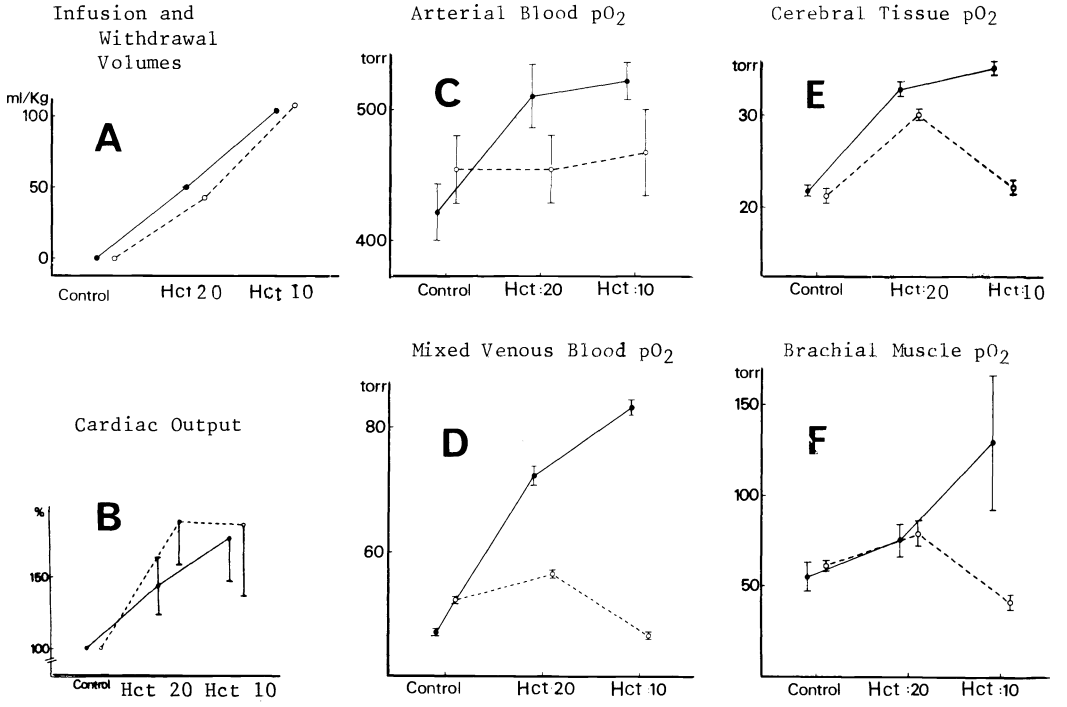


Fig. 7. Comparative effects of normovolemic haemodilution in dogs with Fluosol-DA (•——•) and hydroxyethyl starch solution (o - - - o). "Control" refers to the animals before haemodilution; Hct = haematocrit. From Ref. 102.

of blood was withdrawn (A); note that blood replacement was only partial. Mixed arterial pressure was slightly decreased, and cardiac output (B) significantly increased in both groups in proportion to the severity of the haemodilution, as a consequence of reduce blood viscosity. Arterial blood pO_2 (C) significantly increased during haemodilution with Fluosol-DA at each stage, but not with HES solution. The mixed venous blood pO_2 (D) increased even more (from 48 to 73, then to 83 torr) in Fluosol-DA, while it decreased in the HES solution group when haemodilution became severe. The cerebral tissue pO_2 (E) increased continuously

in the Fluosol-DA test group, while in the HES group it was increased at Hct 20% but then decreased. A similar observation was made concerning brachial muscle pO_2 (F). It was concluded that oxygen transport was well maintained during haemodilution with Fluosol-DA, but not with HES solution, when haemodilution became critical.

A satisfactory blood substitute must also allow for proper transport of CO_2 and pH control. Otherwise acidosis can occur, especially when the elimination of the CO_2 produced in the peripheral tissues is hindered, as is the case for example in haemorrhagic shock. Efficient CO_2 elimination and preserved pH was observed after infusion of PFC-emulsions into dogs having undergone massive haemorrhage (103) or in acute hypolemic accident victims (104).

IV. EXCRETION OF THE PERFLUOROCARBONS

IV.1 Data and methods

One must distinguish : 1) the *intravascular* persistence of the oxygen carrier, which should be as prolonged as possible, and which appears to depend essentially on the characteristics of the emulsion, and 2) its *storage* in the organs *after* leaving the circulatory system until its excretion - no catabolism is observed - which appears to be essentially related to the structure of the PFC itself. Each of these characteristics is described by a half-life, $T_{1/2}$, corresponding to the time necessary for the elimination of half the injected dose of PFC, from the vascular system, and from the organism, respectively.

The life-span of the PFC in the bloodstream is closely dependent on the particle size - the smaller the particles the longer their presence. Particles larger than 0.5 µm are phagocyted and eliminated very rapidly. FC-43 emulsions having an average particle size of 0.09 µm and a half-persistence time in the bloodstream of *ca* 96 hours have been obtained, while this value dropped to *ca* 36 hours when the average particle size increased to 0.2 µm (105). It also depends on the nature of the surfactant (106), but it is not clear whether this is due to some direct action of the surfactant, to its faster elimination, or to differences in particle size of the emulsions.

The intravascular persistence further depends on the dose injected. Thus the intravascular $T_{1/2}$ of Fluosol-DA 20% in man was found to be 7, 14 and 22 hours for doses of 10, 20 and 30 ml/kg body weight respectively (107). Its clinical efficacity for a (small) 300-400 ml dose was estimated as 24 h (97).

Most of the data at present available on the *retention* of the PFCs in the body have been obtained by CLARK's (41,57) and YOKOYAMA's (26,108) groups, using two distinct methods : the first measured, by VPC, the amount of PFC present in the air expired by mice that had been given an intraperitoneal injection of 100 µl of the neat liquid PFC; the second usually analysed the content of PFC in the liver and spleen of rats that received 4 g of the PFC per kg body weight intravenously in emulsified form, but sometimes also measured the expiratory rate of these rats. The values obtained by the various methods are generally different (see for example Table 2) - and may not have the same signification. Thus the half-retention time in the body of *F*-tripropylamine, intravenously injected in emulsified form, was evaluated as 30 days from its disappearance from the organs (26) and as 65 days from its expiratory elimination rate (10).

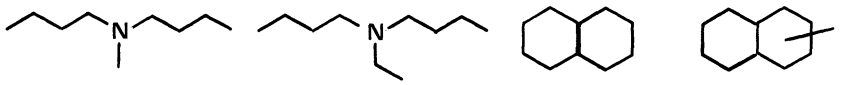
Although the general appreciation of the relative excretability of the PFCs and the general trends observed are certainly meaningful, smaller differences between the figures may not be so. Experimental errors are usually not given, and are difficult to estimate, but may be important, especially in case of PFCs having long retention times. Our own experience with the first method indicates that many factors may influence the excretion rates, including for example the animal's age and level of activity, hence its environment, etc., so that data from *different* sources should only be compared with caution. The second method implies that the various PFCs *distribute* themselves in a similar fashion among the various organs and tissues, which is not certain, and may also depend, not only on the PFCs' characteristics, but on those of the emulsion, as well as on the physical condition of the animal. It has also been shown that the rate of transpiration of *F*-decalin is at least 5 times higher when it is injected intraperitoneally in emulsified rather than in neat *form* (57). Further uncertainty about the meaning of the figures arises from the fact that many of the compounds tested were *mixtures* whose components may have drastically different $T_{1/2}$ even if isomeric (*vide infra*).

The quantitative analysis of fluorine in organs is now being complemented by long-term histological monitoring. It has been confirmed, for example, that *F*-decalin (half excretion rate 6 days) has practically disappeared from the organs after 2 months, while *F*-tripropylamine, the other PFC constituent of Fluosol-DA, remains detectable after one year, although it does not appear to cause any damage - neither acute nor chronic inflammatory reactions - to the cells that host them (8,35,109-110).

IV.2 Trends in the excretion rates of the perfluorochemicals in relation to their structure and other characteristics

The excretion of the PFCs is a *sine qua non;* it is one of the criteria that are universally retained to decree whether a given PFC has any future in blood substitution. Much effort has therefore been devoted to the search for compounds having good elimination characteristics ($T_{1/2} \sim 2$ weeks), and to the recognition of which structural or other features govern this property.

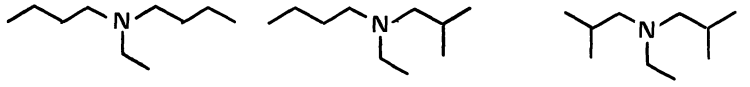
Within a homologous series, the rate of excretion is highly dependent on the molecular weight, as shown by the following examples ($T_{1/2}$) (26) :



(23 days) (420 days) (7 days) (100 days)

(all compounds perfluorinated)

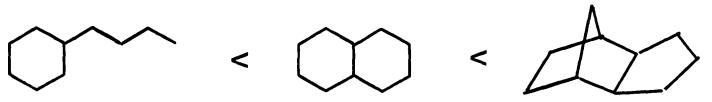
The beneficial effect of ramification on the excretion rate is now also firmly established ($T_{1/2}$) (26) :



(420 days) (150 days) (50 days)

(all compounds perfluorinated)

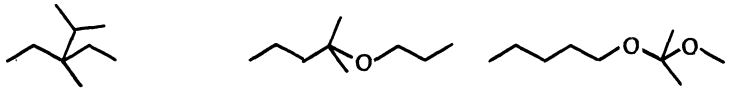
The effect of cyclisation, i.e. increased excretion rates, has been established for a given number of *carbon* atoms, as for example in the series :



$C_{10}F_{20}$ < $C_{10}F_{18}$ < $C_{10}F_{16}$

(all compounds perfluorinated)

but it is not clear to us whether this is due to the cyclisation itself, and to the compactness it confers on the structure (26,41), or whether it simply stems from the decreased *molecular weight*. In accordance with the latter idea one may note for example that the linear F-44E and bicyclic *F*-decalin, with molecular weights 464 and 462 respectively, display very comparable excretion rates (Table 2). The same interrogation holds for the influence of the heteroatoms (which are often thought to be responsible for prolonged retention due to some specific interactions (8, 16, 111)), which increase the retention time when they come in *addition* to the carbon atoms in comparable structures :

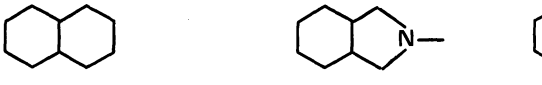


(11 days) [488] (35 days)*[504] (100 days)*[520]

(all compounds perfluorinated)

[Molecular weight]

but decrease it when they *replace* a CF_2 group :



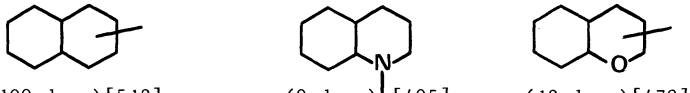
(7 days) [462] v.p. 13 torr

(5 days)*[445] v.p. 24 torr

(4 days)*[428] v.p. 26 torr

*toxic (probably too volatile or containing volatile impurities)

The inverse trend is even found in *F*-methyldecalin :



(100 days)[512] (9 days)[495] (12 days)[478]

With respect to the two characteristics which limit the relevancy of PFCs to blood substitution, i.e. too high volatility, which is a cause of toxicity, and half-retention times longer than two weeks, we feel that present knowledge allows the setting of the *effective molecular weight range* at 460-520. Structural modifications will probably not extend these (narrow) limits by more than 10-20 mass units.

Correlations between excretion rates and various thermodynamic parameters have been sought. As PFCs are excreted by exhalation, it was suspected that their excretion rate might depend on their vapour pressure. Such a general dependence was indeed found, but no precise correlation (10). But vapour pressures, which also depend on molecular weight, are clearly not the sole factor in determining the excretion rate : thus, for example, the compound designated FHQ (*vide supra*) has an expiratory half retention time of 9 days, while that of *F*-ethyl N,N-di-n-butylamine was estimated as 420 days, although they have very close vapour pressures of 8-8.1 torr (but one should again note the large difference in molecular weight : 495 and 571 respectively), and FX-80 has an exceptionally long retention time (several months or longer) in spite of its high volatility (58 mm Hg).

CLARK and MOORE proposed that the expiration rate is linked to the solubility of the PFCs in the lipids, a characteristic which is likely to intervene in the circulation of the PFCs in the body and in their passage through the alveolar membranes (41,57). As a relative measurement of this solubility, they examined the higher critical solubility temperature of a wide variety of PFCs in hydrocarbons (hexane was chosen for convenience), and found a definite correlation within a given homologous series between these temperatures and the transpiration rates of the PFCs administered intraperitoneally in mice. Both parameters were further related to HILDEBRAND's solubility parameter, and hence to the enthalpy of vaporisation of the PFC. The predictive value of these relationships is however limited, being confined within a series of closely related compounds. The fact that *F*-n-butyltetrahydrofuran (FX-80) is excreted only very slowly, when injected intravenously, although it has a critical solubility temperature close to that of *F*-decalin, remains unexplained (18).

Another suggestion has been that some PFCs, especially those containing heteroatoms, combine with cell constituents such as cytochromes to a greater extent than others (18). This would have explained why *F*-tripropylamine is retained longer than *F*-decalin. A number of PFCs were therefore tested against a rat liver microsomal cytochrome P-450 preparation. But the results were, in the case of *F*-decalin and *F*-tripropylamine, the opposite of what would be expected if such an interaction were responsible for the long-term retention, and no correlation was found, either, between the effect of PFCs on cytochrome P-450 when observed, and the presence or absence of a heteroatom (18). The fact that the uncoupling action of PFCs on cytochrome P-450 could be decreased by purification of the compounds sheds some doubt on the origin of this uncoupling; the author himself on this occasion stresses the need to do experiments with as pure compounds as possible.

The detailed mechanism by which the PFCs are excreted is still imperfectly understood. An active transportation of the PFCs through histiocytic cells has been found (35). Since these phagocytes have a definite life span, shorter than the residence time of many PFCs in the body, this probably means that in the case of the longer-retained ones, several generations of phagocytes participate in the wandering and transport of the PFCs from the storage sites to blood and to the lungs, until definitively excreted.

V. OTHER BIOMEDICAL APPLICATIONS OF PERFLUOROCHEMICALS AND RELEVANT SOLUBILITY DATA

V.1 Solubility and transport of drugs and other biologically relevant substances by perfluorochemicals

There is almost no data available yet on the solubility, and possible interactions, of other chemicals and biologically relevant substances than gases in PFCs. Such knowledge is desirable in connection with the *in vivo* transport of metabolites or drugs, many of which in normal circumstances are bound to plasma proteins when conveyed by blood. Whether this transport function might be taken up, and to what extent, by PFCs, remains to be established.

Also of interest would be the possibility of using PFC-emulsions as a vehicle to transport drugs aimed at specific target-organs or tumours. The absence of sensitivity of the PFC-particles - by contrast to red cells - to osmolarity could also permit action on the blood-brain barrier (112). Further applications that are envisaged concern the extraction of specific biomolecules produced for example by cultures of microorganisms (Section V.3).

A preliminary evaluation by VPC of the solubility of a variety of carboxylic acids, amino-acids, sugars, etc., in a range of linear PFCs related to $C_6F_{13}CH=CHC_6F_{13}$, has been undertaken (113). The solubilities of HCOOH, CH_3COOH, CF_3COOH and CCl_3COOH were not affected by the PFCs' being degassed or not, or saturated with O_2 or CO_2; they increase when hydrocarbon segments are introduced into the PFC, especially in terminal position. It can be seen from Table 4 that the solubility of simple carboxylic acids in $C_6F_{13}CH=CHC_6F_{13}$ is far from negligible (80 moles/100 moles or 7 vol.% at 37° for acetic acid), suggesting the formation of dimers through hydrogen bonds, so that their solubility appears to be essentially governed by the hydrocarbon chain. Increasing the acid's chain length provokes a sharp decrease in solubility. This again (as well as low reciprocal solubility) illustrates the profound difference in nature that exists between hydrocarbon and fluorocarbon compounds and the non-ideal behaviour of their mixtures. The substitution of CH_3 by CF_3 in acetic acid leads to

TABLE 4 : Solubility of a variety of carboxylic acids, amino and fatty acids, and oses in $C_6F_{13}CH=CHC_6F_{13}$ (moles/100 moles at 37°C) by GPC (113).

HCOOH (at 50°C)	$5.5(\pm0.2).10^{-2}$	HOOC-COOH	$32\ (\pm3).10^{-4}$
CH_3COOH	$80(\pm3)$	$HOOC-CH_2-COOH$	$22\ (\pm2).10^{-4}$
CF_3COOH (at 70°C)	*miscible*	$HOOC-(CH_2)_2-COOH$	$13\ (\pm1).10^{-4}$
CCl_3COOH (at 70°C)	$0.1(\pm0.01)$	$HOOC-(CH_2)_3-COOH$	$6.3(\pm0.4).10^{-4}$
CH_3CH_2COOH	$71(\pm2.7)$	$HOOC-(CH_2)_4-COOH$	$3.2(\pm0.2).10^{-4}$
$CH_3(CH_2)_2COOH$	$56(\pm2)$	Glycine	$10.5(\pm0.7).10^{-4}$
$CH_3(CH_2)_3COOH$	$39.3(\pm1.6)$	L-Alanine	$8.6(\pm0.5).10^{-4}$
$CH_3(CH_2)_4COOH$	$27.5(\pm1.2)$	L-Phenylalanine	$4.8(\pm0.3).10^{-4}$
$CH_3(CH_2)_5COOH$	$13.2(\pm0.75)$	L-Leucine	$5.6(\pm0.3).10^{-4}$
$CH_3(CH_2)_6COOH$	$5.1(\pm0.2)$	L-Aspartic acid	$5.8(\pm0.4).10^{-4}$
$CH_3(CH_2)_8COOH$	$96.8(\pm2).10^{-2}$	L-Cysteine	$2.8(\pm0.1).10^{-4}$
$CH_3(CH_2)_{10}COOH$	$6.1(\pm0.3).10^{-2}$	L-Methionine	$2.5(\pm0.1).10^{-4}$
$CH_3(CH_2)_{12}COOH$	$1.34(\pm0.07).10^{-2}$	L-Serine	$6.6(\pm0.4).10^{-4}$
$CH_3(CH_2)_{14}COOH$	$0.59(\pm0.02).10^{-2}$	L-Tyrosine	$6.7(\pm0.4).10^{-4}$
$CH_3(CH_2)_{16}COOH$	$0.08(\pm0.01).10^{-2}$	D-Erythrose	$7.5(\pm0.7).10^{-4}$
$CH_2=CHCOOH$	$47(\pm1.8)$	D-Ribose	$11.2(\pm0.4).10^{-4}$
$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ *cis*	$1.18(\pm0.09).10^{-2}$	Dihydroxyacetone	$20.0(\pm1).10^{-4}$
		D-Glucose	$17.0(\pm1.5).10^{-4}$
$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ *trans*	$1.10(\pm0.07).10^{-2}$	β-D-Galactose	$22.0(\pm2).10^{-4}$
$CH_3(CH_2)_3(CH_2CH=CH)_2(CH_2)_7-COOH$	$1.87(\pm0.07).10^{-2}$	β-D-Fructose	$18.1(\pm1.5).10^{-4}$
		C_6H_{14}	$78(\pm2.5)$
$CH_3(CH_2)_3(CH_2CH=CH)_4(CH_2)_7-COOH$	$2.2(\pm0.1).10^{-2}$	C_6H_6	$54(\pm2)$
$CH_3CH(OH)COOH$	$1.77(\pm0.07)$	H_2O	$<5.10^{-2}$

complete *miscibility*. More unexpected, therefore, is the very low solubility found for CCl_3COOH. Formic acid is even less soluble, but can be drawn into solution by CF_3COOH : *ca* equimolar mixtures of HCOOH and CF_3COOH become miscible with $C_4F_9CH=CHC_4F_9$ (Fig. 8). Such co-dissolution effects might prove of interest in drug transport, although their potential might be jeopardized in the presence of an aqueous phase in view of the partition coefficients, which for most polar molecules are strongly in favour of water.

The solubility of long-chain fatty acids, di-acids, amino acids and oses is low but measurable. Even when low, these solubilities may be relevant to drug transport, since *transport* depends both on solubility and on *diffusion* rates. The latter are expected to be high (see Section III.4 for O_2), in view of the very low intermolecular forces present in PFCs, and to result in notable transport of substances through a PFC phase, even for very low solubilities. This is confirmed by preliminary indications obtained on the transport of CH_3COOH and even NH_3 through a layer of PFC (113).

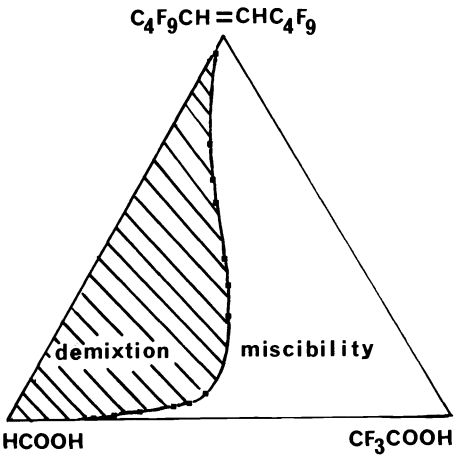


Fig. 8. A ternary PFC/CF_3COOH/HCOOH solubility diagram at 37°; the large extent of the domain of miscibility contrasts with the very low solubility of HCOOH alone in the PFC.

V.2 Perfluoro derivatives as radiopaques

Brominated perfluoroalcanes $C_nF_{2n+1}Br$, with n = 6-8, have been successfully used for contrast enhancement in diagnostic radiology, either in the neat state or in emulsified form. The iodides and the polybrominated derivatives that have been tested so far in order to obtain better radiopacity proved to be too unstable and/or toxic to be used in biomedical applications. The more volatile *F*-hexylbromide (v.p. 98 torr at 37°C) is preferred for use in the lungs, gastrointestinal tract and lymphatics, but is contraindicated for the intravascular spaces and fluid-filled spaces of the central nervous system, where $C_8F_{17}Br$ is preferred. The latter has been administered intravascularly in the form of rather coarse emulsions; the PFC is then rapidly concentrated in the macrophage cells, resulting in radiopacification of some organs, such as the liver and spleen. One of the most promising and unique characteristics of $C_8F_{17}Br$ is its tendency to concentrate in the macrophages of malignant tumours, thus allowing the diagnoses of tumours as small as 2-3 mm, for example in the brain. Very concentrated, viscous preparations, intended for example to coat the bronchi, are sometimes utilised (114).

The *F*-alkyl bromides appear to be less irritating and safer than most currently utilised radiopaque material. Gasteroenterography, bronchography and alveolography have been performed for several years with such radiopaque fluorocarbon derivatives, both in experimental animals and in human subjects, apparently without adverse effects. They cannot, of course, compete in cost with barium sulphate, but might find applications in those specific circumstances where barium sulphate is contraindicated, for example when there is a risk of leakage of the contrast material into the peritoneal cavity, or for use in patients with hypersensitivity to iodine-containing contrasting agents (114).

V.3 Perfluorochemicals as oxygen carriers in the culture of microorganisms (and the reciprocal use of cell cultures to test them)

Several patents recommend the use of PFCs as oxygen carriers to stimulate the growth of aerobic microorganisms (115-119).

Bis(*F*-hexyl)ethene has for example been used to supply oxygen to lymphoïd cell cultures of the Namalva strain destined for the production of interferon (119). High yields in cell cultures have been obtained, but probably more interesting is the fact that precise control of the O_2 and CO_2 supply and pH, and smooth, undamaging agitation of the culture, could be effected via the underlying layer of PFC; there is no longer any need for a supernatant atmosphere. If these advantages are confirmed, it could lead to the development of new technology in fermenters designed for mass culture of microorganisms producing vaccins and other biological substances of interest.

Advantage can also be taken of such procedures each time it is desirable to prolong the lifespan of microorganisms, tissues or other living material that is confined in a limited space, as for example while under observation in the sample cell of a spectrometer, with little room - or technical difficulties - to assure proper entry of gaseous oxygen.

Conversely, strain L mouse fibroblasts (9) and Namalva (120) cell cultures have been used as a means of checking the atoxicity and purity of the PFCs and other components that make up the blood substitutes. The effect of the material on the viability, morphology and multiplication rate of the cultures is controlled. Such tests provide highly sensitive and efficient tools to ascertain the innocuousness of the material, and thus to monitor the effectiveness of a given purification step, hence to help establish reproducible, efficient, economical, industrially feasible standard protocols for purifying and detoxifying PFCs and other material for biomedical use. The urgent need for such standard protocols has been stressed repeatedly (7,9,18).

VI CONCLUSIONS

When summarizing our appreciation of the research on PFC-based blood substitutes at its present stage, we come to the following conclusions - although some of the problems invoked may rapidly reach a solution in view of the fast evolution the field is experiencing :

1) There is little chance of finding new PFC O_2-carriers having much higher O_2-dissolving capacity : a limit has probably been reached here. Fortunately this is not the sole factor that determines O_2-transport and - even more important physiologically - O_2-*delivery*. The ability of PFC-emulsions to deliver oxygen *in vivo* is indeed enhanced by several favorable factors : When compared to blood, the lower affinity of PFCs for O_2, and their linear increase in O_2-content with pO_2, permit the attaining of considerably higher arterial pO_2 (which can thus be controlled in a new way), hence much higher diffusion rates, allowing better extraction of O_2 by tissues. Lower viscosity permits higher cardiac output, hence higher oxygen-flux, as well as better utilisation of the peripheral microcirculation.

2) Although it is now established that most PFCs are innocuous when pure, and when within certain physical norms, there is no doubt that more research needs to be done to verify the absence of any long-term side effects and in order to better evaluate the benefit *vs* risk balance for the patients.

3) As our knowledge of the "physiology" of the PFCs and of the differences in behaviour among them, especially with respect to toxicity and excretion, becomes deeper, the importance of using pure, well-defined PFCs becomes more manifest, particularly in view of the magnitude of the doses the patients receive. In case the use of mixtures of PFCs cannot be avoided, it is imperative that all of their components be identified and that the innocuity of each one be individually secured.

4) With the prospect of large-scale commercialisation coming closer, the problem of the industrial feasibility of both the preparation and the purification of the PFCs, which was often neglected in the screening stages, becomes more acute.

5) Compounds that have acceptably short retention times in the body have now been found. But the exact mechanism of their excretion has yet to be clarified; labelled PFCs would be very helpful in this respect.

6) Long-term stability has not yet been achieved with the present emulsions, and remains the most central problem. The significant increase in stability observed in the classical emulsions upon adjunction of yolk phospholipids is certainly an encouragement to devote more efforts to the screening of new formulations using other surfactants, including perfluorinated ones, in view of optimising this characteristic. The alternative way, employing *micro*emulsions, has received little attention so far, and is now starting to arouse interest. The obtaining of bio-compatible microemulsions having a large domain of stability - which must include pure water - would indeed be the ideal answer to the need for an increase in the long-term conservation of PFC-based blood substitutes.

The clinical tests performed so far on Fluosol-DA - the first PFC-based blood substitute to be used on humans - have proved both its innocuity - at least short-term - and beneficial effects, in spite of its weaknesses, among which are its insufficient stability, necessitating its transport and storage in the frozen state, the overlong retention of *F*-tripropylamine in the organs and the insufficient purity of the latter. The dose above which the use of Fluosol-DA becomes beneficial in comparison with non-O_2-carrying plasma substitutes, and the cases in which its use results in a positive benefit *vs* risk balance for the patient, remain to be ascertained more precisely. Room for improvement also comprises the preparation of emulsions devised for specific uses : the recuperation of anoxic tissues and organs, for example, requires that more consideration be given to the rheologic qualities of the emulsion than to their long-term stability; this on the other hand is the main goal when the emergency market is aimed at.

PFC-based blood substitutes will in our opinion not replace transfusion of natural blood, but:

1) They will *complement* it in helping to cope with emergency situations - on-site rescue as well as mass casualty situations - or unforeseen shortage, to preserve isolated organs and limbs, and in extra-corporeal circulation, as well as to improve conservation of natural blood, which could then be employed more judiciously, especially for the separation (and specific uses) of its components, whose functions can by no means be performed by these substitutes. From this standpoint, the development of blood-substitutes will help the blood banks in their mission both by allowing better employment of blood resources and by providing them with a new means of saving life.

2) In addition, the PFC-based blood substitutes will offer a range of totally new therapeutic indications and benefits to medicine, *different* from those of blood. The conjunction of their lower viscosity and particle size with the possibility of attaining higher arterial pO_2, O_2-diffusion rates and O_2-extraction by the tissues and organs, appears to be particularly promising in the treatment of diseases or trauma, which involve microcirculation disturbances, as in haemorrhagic shock, anoxia and ischemia, including heart infarct, cerebral vessel diseases or extended burns.

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