

# Oral administration of factor VIII

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*Factor VIII oral*Oral Administration of Factor VIIIReport of first consultant visit to G.C.C. and  
suggestions for future research§1 Introduction

Our stay at Green Cross Corp. in Osaka has in our opinion been very useful for the project of F.VIII loaded liposomes for a variety of reasons. Before going into detail we want to thank G.C.C. and all its individual members for the efficient cooperation and open discussions on the scientific level as well as for the magnificent hospitality and extremely enjoyable social and cultural features included in our program.

The main profit for the oral factor VIII project is that we now have a clear insight in the possibilities and limitations of the G.C.C. research laboratory, because we actively participated in the laboratory work. It is clear that at this moment there are no difficulties in:

- a) The general procedure of making liposomes and the preparation of phospholipids.
- b) The estimation of factor VIII in plasma, in concentrates and in liposome preparations.

Minor details may have to be adjusted, but in these we may be easily consulted via written communications. We understood that, by the kind cooperation of clinicians in the Osaka area there are ample possibilities for experimental trials of oral preparations in hemophiliacs.

However, already at this point we want to warn for too early and too quick jumping to clinical trials because this will inevitably lead to a high rate of failures with concomitant disappointment and stress on both the patient(s) and the investigator(s).

This brings us directly to the crucial difficulty in this project: We do not as yet know how to reproduce successful oral administration of liposome entrapped Factor VIII.

It has been shown both in Japan and in the Netherlands that, in principle oral administration is possible. However, uncontrolled circumstances make that more often than not oral administration results in no rise of plasma factor VIII activity. To find the circumstances that make oral administration routinely feasible must be the aim and object of future research.

Four sets of variables determine the success of oral factor VIII administration:

- α. Successful entrapment of factor VIII in liposomes
- β. Resistance of liposomes to gastric and duodenal milieu, including pancreatic enzymes and bile acids
- γ. Uptake of liposomes or liposomal derivatives by the gut wall.



d. As yet unknown factors

- d<sub>1</sub>. After transport into the portal circulation
- d<sub>2</sub>. Peculiarities of the individual patient  
(inhibitors, conditions of gut and/or R.E.S. etc.)

During our stay in Osaka we conducted a series of experiments meant to start the solution of these problems at the same time as bringing the work in Osaka and Maastricht under a common denominator. As it was already known that all oral administrations in which the patient did not have a completely empty stomach have been a failure, it was decided to try and repeat precisely our first experiments as published in "the Lancet." Also it was known that Profilate, and under certain circumstances also Concoeight give poor entrapments. Earlier we found that dialysis improves entrapment. Therefore at the first day of work it was found out under what conditions entrapment could be improved. Dialysis against phosphate buffer appeared to offer the best conditions. (Here, and in other places indicated with an asterisk (\*) we refer to the notebooks of the Laboratory staff for experimental details)

It should be noted that entrapment of Profilate after dialysis against phosphate buffer is still way below the entrapment we found with the Swiss Red Cross (S.R.C.) material in our earlier experiments (>60%).

The next day phosphate-dialysed Profilate was administered orally to Mr. Nakamura, but no effect whatsoever could be found. This reinforced our conclusion that high entrapment is a prerequisite for successful oral administration.

## §2 How to obtain high entrapment

Entrapment of factor VIII is dependent on both the composition of the phospholipid used<sup>(a)</sup> and the composition of the factor VIII concentrate employed (b).

ad a Extensive experiments have been done on the influence of lipid composition on entrapment. The effect of the relative concentration of phosphatidic Acid (P.A.) as well as the use of pure or mixed phospholipid (P.L.) preparations have been reported by Mr. Fukushima. Attention should still be paid to the importance of phosphatidyl serine (P.S.) for entrapment. For example we noticed by two dimensional thin layer chromatography that P.S. is present in yolk P.L. which might explain the better uptake of factor VIII by this material than by pure phosphatidyl choline (P.C.).

It is of the utmost importance to realize that the P.L. preparation has to meet two distinct requirements.

1) high entrapment and 2) resistance to enteric degradation. These two may point in different directions. e.g. A high percentage of P.A. facilitates entrapment but decreases the shell number of liposomes, thus enhancing enteric degradation.



ad b It appears that successful entrapment is very much dependent upon the factor VIII concentrate used. We found that S.R.C. material is superior over Kabi preparations and that Concoeight is better than Profilate. This suggested the following experiments:

1) Investigate the influence of fibrinogen and other proteins on entrapment. The first experiments are being carried out at this moment (\*). They concern fibrinogen. It remains to be investigated whether other proteins present in the concentrate do have any influence. We suggest that this be investigated in detail by the Lab. in Osaka.

2) Investigate the influence of the state of activation of factor VIII. It is known that some factor VIII concentrates show a high potency in vitro but do not cause a high blood level of factor VIII when infused in a hemophiliac. Also it is known that factor VIII has to be activated by thrombin before it can take part in the intrinsic factor VIII activating enzyme, which is its biological function. Binding to P.L. is essential for this biological function. It is therefore not far fetched to conjecture that activation of factor VIII will facilitate entrapment.

This is corroborated by the preliminary results of the electron microscopy (E.M.) of factor VIII loaded liposomes. It appears that here exist two forms of entrapment.

- i: Bulk entrapment of protein solution in large spaces between P.L. multilayers as seen in E.M.
- ii: Entrapment by binding to P.L. not seen in E.M. but to be inferred from the well known hydrophobic binding of factor VIII (compare van Dieyen e.a. J.B.C. to be published in 1981)

It stands to reason that only that part of factor VIII that is intimately bound to lipid can be taken up via the intestine. Low entrapment probably means bulk entrapment only. This explains why clinical trials with low entrapment material are bound to be unsuccessful. Apart from that, the rise in blood level that can be expected when only low amounts of factor VIII are entrapped is minor anyhow.

It will be necessary to carry out controlled activation of factor VIII preparations and to investigate its influence on entrapment. These experiments require sophisticated enzyme kinetic and coagulation setups, the knowhow for which is available in Maastricht. The activation should be carried out with ultrapure thrombin preparations devoid of any protein C activity; preferably it should be coupled to an insoluble carrier so as to make its instant removal possible. The activation of factor VIII should be checked in a pure system by means of tests on chromogenic substrates in order not to be misled by artifacts. Preliminary experiments carried out us in the G.C.C. laboratories show that it is in principle possible to obtain a sufficiently stable activated factor VIII preparation. We suggest that these experiments should be continued and extended in Maastricht by one or more G.C.C. collaborators.



These experiments also pertain to what might become a highly important sideline of the research on oral factor VIII viz: The preparation of material with Factor Eight Inhibitor Bypassing Activity (F.E.I.B.A.).

Preliminary experiments in our laboratory indicate the possible nature of FEIBA production. In this respect also administration to Von Willebrandt patients might be interesting.

### §3 How to prevent enteric breakdown

The interesting idea and the reported success of enteric coating requires our closest attention. It must not however detract from the search for optimally stable liposomes because the following practical difficulties seem to exist.

i) Enteric coating is carried out after first freeze-drying the liposomes. This means that the liposomes are to be reconstituted in the duodenum by uptake of the fluid available there. Bile salts, proteolytic and lipolytic enzymes contained in this fluid will therefore be incorporated in the liposomes and quickly destroy them.

ii) Enteric coated capsules containing material with a low amount of entrapped factor VIII have to be given in large quantities. These will not pass the pylorus all at once. The degradation of the first few capsules in the duodenum will stimulate the secretion of bile salts and enzymes. Therefore the remaining capsules will meet highly unfavourable conditions in the duodenum. We fully understand that enteric coating is a matter concerning G.C.C. but we thought it advisable to bring these drawbacks to your attention. The more so as there exists various possibilities to circumvent them.

We suggest:

a) In vitro experiments in simulated duodenal fluid containing trypsin(ogen), (pro)phospholipase etc. (i.e. not the simulated duodenal fluid from the pharmacopeia), and investigation of the inhibition of its liposome-degrading activity by Soybean Trypsin Inhibitor (S.B.T.I.) or other inhibitors. S.B.T.I. will not only inhibit the breakdown of factor VIII by trypsin but also prevent the activation of phospholipase A<sub>2</sub>. The same experiments will have to be carried out with duodenal fluid from a healthy individual.

b) In vivo experiments of administration of factor VIII loaded liposomes with or without S.B.T.I. via a duodenal tube in volunteer hemophiliacs.

c) Investigation of the use of sphingomyelin (S.M.) as a P.L. carrier for factor VIII. S.M. is not degraded by intestinal enzymes and has a high resistance to bile salts. The experiments mentioned sub a and b above should be repeated with these liposomes. Also the possibility should be investigated to use the ghosts of ruminant erythrocytes (e.g. cow, sheep) as a cheap source of S.M.-rich material.



Preliminary experiments with S.M., as compared to yolk PL and PC gave the following results (\*).

	super- natant	lipo- somes	yield	pH	temp.
YPL	73	27	100	6.68	37°C
PC	80	20	100	6.64	37°C
SM	28	59	87	6.73	40°C

*Rokushima's*

d) Dr. ~~Fujikawa's~~ interesting suggestion of anal administration of factor VIII may well be pursued.

In view of our extensive expertise in enzymology we suggest that a and c be carried out by G.C.C. personnel in Maastricht; b and d are of clinical nature and can be carried out better in Osaka.

#### §4 Uptake by the intestine

No experiments have as yet been done in this respect. Preliminary experiments have been suggested both in Osaka and Maastricht. We have some suggestions as to the protein that might facilitate enteric resorption of liposomes. We suggest that the Osaka and Maastricht experiments will be carried out by Maastricht personnel. We already have some experience with transport of biologically active peptide hormones. These experiments are beyond the contract with G.C.C. however.

#### §5 Uptake by the R.E.S., antigenicity

The observation of an unexpectedly long half-life time after oral administration of factor VIII suggests the formation of an as yet unknown pool, possibly originating from uptake by and release from the R.E.S. In order to investigate this further we are planning to infuse factor VIII loaded liposomes in hemophilic dogs. Also the use of resealed erythrocyte ghosts as carriers for intravenous factor VIII administration is planned. The antigenicity of factor VIII loaded liposomes is of course a matter of extreme practical importance and should be one of our major concerns. We plan to study these aspects in Maastricht. Again we propose to proceed independently and join forces with G.C.C. when a more detailed planning is possible.

#### Summary and conclusions

- Our visit to the G.C.C. research laboratories has given us a clear insight in the possibilities of these laboratories and therefore enables us to do detailed suggestions as to further research.
- Several experimental problems have been sorted out during our work in the lab. (\*)

Factor VIII oral

- Elaborated proposals are done for further experiments that will show
    - a) How to obtain high entrapment
    - b) How to prevent enteric breakdown
- These experiments require the presence of one or more G.C.C. personel in the Maastricht laboratory for a period of not shorter than three months.
- We make global suggestions for further experiments on enteric and/or R.E.S. uptake of factor VIII and on studies of the antigenicity of orally administered proteins. These experiments can be carried out independently in Osaka and Maastricht. Closer co-operation can be decided upon later.
  - It is likely that as a byproduct of the present studies materials can be developed with Factor VIII Inhibitor Bypassing Activity. Study of such material is not included in our contract. Consultants give G.C.C. the option to extend the contract in this direction, to be decided within 6 months.

Osaka 18 December 1980

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