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Stability of insulin mixtures in disposable plastic insulin syringes

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The miscibility of short and long acting porcine, human and bovine insulins has been investigated using HPLC. Soluble insulin is rapidly lost from solution when mixed with both isophane and zinc insulin, although the mechanism may be different in these two cases. The time for up to 50% or more to be lost is often less than 2 min. The percentage lost is dependent upon the ratio of the mixture and the type and origin of the insulin. In the case of soluble : zinc mixtures it is greatest for porcine and least for bovine, in the case of soluble : isophane mixtures it was least for human and greatest for bovine. The greater the original amount of soluble insulin present the less the loss. The results help explain recent clinical reports that mixtures of soluble and zinc insulins fail to achieve satisfactory control of post-prandial blood sugar levels.

Modern insulins can be classified by origin—bovine, porcine or human, and by duration of action—fast, intermediate or slow acting (Larner 1980).

Since the 1970s twice daily injections of a mixture of insulins have become the usual method of diabetic management (McPherson & Feely 1983). Short and intermediate acting insulins are usually mixed in the syringe immediately before injection. The ratio between each type of insulin is considered to be critical in the effective control of diabetes.

The introduction of plastic, disposable, insulin syringes has simplified the injection process and there is potential benefit in having the daily dose pre-measured and stored in syringes. However, post-prandial control of blood sugar is difficult to achieve with a twice daily regime, particularly after breakfast (Schmidt et al 1979). Furthermore, Mulhauser et al (1984) found a reduction in serum insulin and glycaemic control when soluble and ultra lente zinc insulins were mixed before injection, as did Heine et al (1984, 1985) with soluble and insulin zinc suspension insulins.

We have examined the stability and degree of interaction of a range of bovine, porcine and human short and longer acting insulins when mixed in the same syringe.

MATERIALS AND METHODS

Materials

Standard commercial vials of the following U100 insulins were used: Soluble neutral: Velosulin (Nordisk), Actrapid, Human Actrapid (Novo), Humulin S (Lilly), Neusulin (Wellcome), Quicksol (Boots). Isophane: Insulatard (Nordisk), Humulin I (Lilly), Neuphane (Wellcome), Human Protophane (Novo), Monophane (Boots). Insulin zinc: Monotard, Human Monotard (Novo), Humulin Zn (Lilly), Neulente (Wellcome), Tempulin (Boots).

The same batch of each insulin was used throughout each study.

The syringes were Plastipak 1 mL 100U (Becton Dickinson).

Methods

Mixtures were prepared by adding regular, soluble insulin from one manufacturer in varying ratios to a longer acting insoluble insulin (zinc or isophane) from the same manufacturer. The soluble insulin content was then determined by HPLC after filtering the mixtures through a Minisart 0·22 μm filter and suitably diluting them. Hence any loss of regular, soluble insulin from the mixture and the length of time over which any interaction occurred could be determined.

The method of Adams & Haines-Nutt (1986) was used. This is an HPLC method which consisted of: pump: Applied Chromatography Systems 750/04; column: 3 μm ODS mini-column (3 cm × 5·2 mm i.d.) (Perkin Elmer). Solvents: for porcine and human insulins: 78 : 27·5 ammonium sulphate 0·1 M/0·005 M tartaric acid buffer to pH 3 with sulphuric acid–acetonitrile. For bovine insulins: 78 : 22 ammonium sulphate 0·1 M/0·005 M tartaric acid buffer to pH 3–acetonitrile. Cetrimide, to a final concentration of 0·002%, was added to both solvents.

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Insulin content was determined by peak height measurement which was shown to give a linear response up to and including 80 units mL^{-1} ($r = 0{\cdot}98$). Immediately before analysis the supernatant content of each syringe was diluted five-fold with the appropriate solvent. Insulin standards and insulin mixtures were diluted with water to determine the soluble insulin content. The total soluble and long acting insulin content in the mixture was measured by dissolving in 0·01 M hydrochloric acid. No soluble insulin was detected in the supernatants of any of the insoluble insulins.

Any decrease in regular, soluble insulin was determined using a standard made from the same batch of soluble insulin from which the mixture was prepared. The percentage of soluble insulin remaining was calculated by assuming that, at the time of mixing, the mixture contained 100% of the added amount of soluble insulin, since the mixtures and standard were prepared using the same batches (assuming the soluble insulin content in every vial of the same batch to be identical). The assay results are based on chromatographically recoverable insulin and not biological activity. All analyses were made in duplicate.

To ensure that there was no loss of soluble insulin due to interaction with the syringe or its components the soluble insulins studied were stored for 29 days at 4 °C and room temperature (20 °C). No significant loss of insulin was found.

RESULTS

Porcine

Neutral soluble and isophane insulins (Velosulin + Insulatard, Nordisk). Syringes of mixed insulins were initially assayed after 3 days. After being filtered off, the supernatant was allowed to warm to 20 °C before it was diluted. The detectable soluble insulin component was significantly decreased (Table 1), but showed no further decrease over the following 5 days. However, the total insulin content of the syringe did not change over the 8 days, indicating the decrease in soluble insulin was not a result of degradation or adsorption onto the syringe but due to an interaction with the insoluble component of the mixture.

Table 1. Detectable soluble insulin present in mixtures of soluble (Velosulin) and isophane (Insulatard) insulins 3 days after mixing, expressed as a percentage of the initial soluble insulin content.

Ratio of V : I	Temperatures at which syringes maintained °C	Soluble insulin % detected
3 : 7	4	55
	20	54
2 : 3	4	70
	20	69
1 : 1	4	78
	20	78

The change in the composition of mixed insulins having been found to occur within 3 days, the experiment was repeated with attention directed towards the first 90 s of mixing.

The reaction occurred rapidly, in most cases taking 60–90 s to reach equilibrium (Table 2). The amount of soluble insulin remaining agreed well with the results in Table 1.

Table 2. Detectable soluble insulin present after 30, 60 and 90 s in mixtures of soluble (Velosulin) and isophane (Insulatard) insulins expressed as a percentage of the initial soluble insulin content.

Ratio of V : I	Soluble insulin % detected 30 s	60 s	90 s
3 : 7	69	54	54
2 : 3	76	69	69
1 : 1	82	80	80

Two Nordisk mixed insulins (Mixtard, Initard) with ratios of 3 : 7 and 1 : 1 (soluble : isophane insulins) were assayed chromatographically and were found to contain 54 and 65% of the declared quantity of soluble insulin. The 3 : 7 mixture (Mixtard) contained the same amount of soluble insulin as our own mixtures (Table 1), and the 1 : 1 (Initard) contained a still lower amount.

Neutral soluble and insulin zinc suspension (Actrapid + Monotard, Novo). The change in composition with time for this mixture of insulins was studied using the ratios 2 : 1, 1 : 1, 2 : 3 and 3 : 7 (soluble : zinc insulin). No further loss of soluble insulin occurred after 2 min. All measurements were taken after 5 min and the results are shown in Table 3. The time

Table 3. Percentage of detectable soluble insulin remaining after 5 min in Actrapid : Monotard mixtures, filtered and unfiltered.

Ratio	Actrapid + Monotard	Actrapid + Filtered Monotard	Monotard + Actrapid	Filtered Monotard + Actrapid
2 : 1	56	97	51	92
1 : 1	41	56	36	50
2 : 3	8	27	2	21
3 : 7	4	11	1	4

taken to reach equilibrium (approx. 120 s) was longer than that for the soluble–isophane mixtures. For all four ratios the insulin composition was further measured 10 min after mixing and did not change. In all four ratios soluble insulin was present at the end of the interaction, indicating that any excess zinc initially present in solution was insufficient to allow the reaction to go to completion. The smallest amount was in the 3 : 7 mixture and the largest in the 2 : 1 mixture. For the 2 : 1 mixture the soluble insulin content was 57% of that expected. Grootendurst et al (1983) reported similar results when soluble and insulin zinc suspension were mixed together.

The effect of the zinc insulin complex and the method of mixing on the content of soluble insulin was also examined. The insulin zinc suspension was filtered and the clear supernatant added to the soluble insulin. The soluble insulin content was determined after 5 min. The effect of removing the zinc insulin complex before mixing was a smaller loss of soluble insulin (Table 3). Addition of Monotard to Actrapid gave a smaller loss of soluble insulin than when Actrapid was added to Monotard. With the Actrapid : Monotard (2 : 1) ratio, addition of Monotard supernatant to Actrapid caused a slight turbidity which rapidly disappeared on continuous addition of the remaining Monotard. No opalescence was seen on standing. For all ratios soluble insulin remaining was less if it had been added to the insulin zinc suspension. In view of the findings all subsequent studies were conducted by adding the insulin zinc or isophane preparation to the neutral soluble insulin.

Human

Neutral soluble and isophane insulins. (Human Actrapid + Human Protophane, Humulin S + Humulin I, Novo, Lilly). The interaction between regular soluble insulins and isophane insulins was complete 90 s after mixing (Table 4). The content of soluble insulin was in close agreement with the results found when porcine soluble and isophane insulins were mixed.

For both sets of insulins, the addition of the supernatant obtained by centrifuging the isophane insulin did not result in any decrease in the soluble insulin component 2, 5, 60 min and 24 h after mixing indicating that there is either no excess protamine present in solution in the protamine insulin or that the conditions of mixing are not conducive to the formation of protamine insulin, even if excess protamine is present.

Neutral soluble and insulin zinc suspension (Human Actrapid + Human Monotard, Humulin S + Humulin Zn, Novo, Lilly). The reaction of these human insulins after being mixed took much longer than the porcine insulins to reach completion, taking up to 1 h for the 1 : 1 and 2 : 3 mixtures and up to 2 h for the 3 : 7 Humulin mixture. Results 1 h after mixing are presented in Table 4. The soluble insulin detected after completion of the reaction is much greater for the human insulins than for the porcine.

Bovine

Neutral soluble and isophane insulins (Quicksol, Monophane, Boots, Neusulin, Neuphane, Wellcome). The soluble insulin content at equilibrium when these insulins were mixed is presented in Table 4. The reaction reached completion within 2 min.

It can be seen from these results that more soluble insulin is lost when bovine insulins are mixed than when similar porcine and human insulins are mixed. The supernatants from the isophane insulins were mixed with the neutral soluble insulins and the soluble insulin, determined after 2, 5, 60 min and 24 h. There was no decrease in the soluble insulin content at any of these times.

Neutral soluble and insulin zinc suspension (Quicksol, Tempulin, Boots; Neusulin, Neulente, Wellcome). The soluble insulin remaining when these are mixed is shown in Table 4. These results were obtained after 1 h but as they varied so markedly from those with previous mixtures, the experiment was continued for up to 8 h with no further changes observed.

Table 4. Percentage of detectable soluble insulin in commonly used mixtures at equilibrium.

Type of insulin	3 : 7	2 : 3	1 : 1	2 : 1
Velosulin : Insulatard	52	69	81	
Human Actrapid : Human Protophane	55	70	80	
Humulin S : Humulin I	55	69	80	
Quicksol : Monophane	41	49	72	
Neuphane : Neusulin	38	52	68	
Actrapid : Monotard	4	8	41	56
Human Actrapid : Human Monotard	19	56	85	94
Humulin S : Humulin Zn	37 (1 h) 19 (2 h)	56	83	94
Quicksol : Tempulin	100	100	100	
Neusulin : Neulente	56	100	100	

DISCUSSION

Our results show that porcine and human soluble and zinc suspension insulins react to an appreciable extent. This is in agreement with the findings of Grootendurst et al (1983).

The reaction occurs rapidly for porcine insulins in

the 3:7 and 2:3 ratios when virtually no soluble insulin remained after 75–80 s. Grootendurst et al (1983) found similar results over a longer time, as did Nolte et al (1983). The in-vivo work of Berger et al (1982) with porcine insulins suggests that an immediate injection of a mixture of neutral soluble with insulin zinc suspension insulin results in an unaltered absorption pattern of the soluble component, whereas injection 5 min after mixing resulted in a significant delay in the rise of circulating levels. Berger et al used a 1 : 1·6 mixture. Our results with a 1 : 1 mixture show a slower reaction than for the 3 : 7 or 2 : 3 ratios (Table 5) which may account for these in-vivo

Table 5. Detectable soluble insulin present up to 2 min after mixing in mixtures of soluble (Actrapid) and insulin zinc suspension (Monotard) expressed as a percentage of the initial soluble insulin content at 20 °C.

	% Soluble insulin detected		
Original ratio (A : M)	3:7	2:3	1:1
Time (s)			
30	15	30	74
60	8	20	46
90	4	12	42
120	4	8	41

observations, particularly as the results using the 3 : 7 and 2 : 3 mixtures show that within 30–60 s of mixing the soluble insulin has complexed with the free zinc ions in the suspension to an appreciable extent.

These results have implications for the many diabetics who pre-mix insulins before injection and give an analytical base to the clinical findings quoted in the introduction (Schmidt et al 1979; Mulhauser et al 1984; Heine et al 1984, 1985). When human insulins (Human Actrapid + Human Monotard and Humulin S + Humulin Zn) were studied under similar conditions the reduction in soluble insulin component was less marked. Furthermore, bovine insulin mixtures (Quicksol, Tempulin, Neusulin, Neulente) showed no reduction in soluble insulin content under the same conditions for all but one mixture (3 : 7 Neusulin : Neulente).

The preservatives used in the soluble and zinc suspension insulins vary as is shown in Table 6. Schlichtkrull et al (1975) have noted that phenol alters the crystalline structure of insulin zinc suspension complexes and should not be used in such preparations, parabens being the preservative of choice. For those mixtures where phenol or *m*-cresol are not present, the loss of soluble insulin was considerable, i.e. Actrapid + Monotard and Actrapid + Rapitard. For those mixtures where phenol and/or *m*-cresol are present in both insulin preparations, i.e. Quicksol + Tempulin, the soluble insulin content remained at 100% of all ratios studied. For those mixtures containing parabens and phenolic preservative, the soluble insulin component shows a small loss. These results indicate that phenol and *m*-cresol may decrease the loss of soluble insulin when insulin zinc suspensions and soluble insulins are mixed, possibly as a result of phenol's action on crystal formation. Thus the type of preservatives used in the various brands of insulin would appear to influence the degree of interaction between the soluble and longer acting insulins.

With the exception of Humulin S and Human Actrapid, all the insulins studied are buffered by sodium acetate or sodium phosphate (Table 6) and

Table 6. Insulins and their preservatives.

Insulin	Preservatives	Buffers
Actrapid	Parabens	Acetate
Human Actrapid	Phenol	No buffer
Human Monotard	Parabens	Acetate
Human Protophane	*m*-Cresol + phenol	Phosphate
Humulin I	*m*-Cresol + phenol	Phosphate
Humulin S	*m*-Cresol	No buffer
Humulin Zn	Parabens	Acetate
Initard	*m*-Cresol + phenol	Phosphate
Insulatard	*m*-Cresol + phenol	Phosphate
Mixtard	*m*-Cresol + phenol	Phosphate
Monophane	*m*-Cresol + phenol	Phosphate
Monotard	Parabens	Acetate
Neulente	*m*-Cresol + phenol	Acetate
Neuphane	*m*-Cresol + phenol	Phosphate
Neusulin	*m*-Cresol	Acetate
Quicksol	*m*-Cresol	Acetate
Tempulin	*m*-Cresol + phenol	Acetate
Velosulin	*m*-Cresol	Phosphate

although it is possible that the nature of the buffer could alter the readiness with which soluble and longer acting insulins interact, there appears to be no correlation between our results and the buffer system used. We found that by increasing the soluble insulin component in soluble : insulin zinc suspension porcine mixtures, a smaller loss of soluble insulin is observed and this might therefore be expected to improve diabetic control, particularly postprandially.

The soluble and isophane insulins investigated (Bovine, Porcine and Human) also reacted with each other although the loss of soluble insulin was least with human and greatest with bovine insulins.

The ratio of porcine soluble insulin complexing with the isophane insulin originally present is constant (approximately 1 to 5). Thus, assuming there is sufficient soluble insulin to enable the reaction to go to completion and sufficient isophane insulin to take up the available soluble component, the amount of

soluble insulin remaining may be predicted for any combination of soluble and isophane insulins (Velosulin: Insulatard). This at first seems at odds with previously published work (Grootendurst et al 1983) where it was found that there was no decrease in the soluble insulin content. The method of analysis used by Grootendurst et al, however, depends upon a turbidometric assay which required filtration of the isophane component before addition of the soluble insulin. We repeated this by centrifuging the isophane insulin before adding it to the soluble insulin. The resulting supernatant solution containing the soluble component was then assayed. This showed that the soluble insulin content had not decreased. This is in agreement with Grootendurst's results and shows that the loss of soluble insulin from the mixture is not due to any excess protamine that may be present in solution but due to the protamine insulin complex itself, since, when it is removed by centrifugation before mixing, there is no decrease in the total soluble insulin content. The results of Grootendurst et al (1983) for mixing soluble and isophane insulin supernatants are, therefore, artefacts. Our results show that there are significant changes when such insulins are mixed immediately before injection. The nature of this binding between neutral soluble porcine insulin and isophane porcine insulin is uncertain, but it appears to be a reversible chemical interaction, for the in-vivo studies of Berger et al (1982), Kolendorf et al (1978) and Heine et al (1984, 1985) demonstrate that, once the insulins are injected, the absorption kinetics of the mixture (Velosulin + Insulatard) are identical to that observed when the two insulins are injected separately into different sites, i.e. the full effect of the regular, soluble insulin is seen immediately upon injection. Chromatographic analysis of commercially prepared soluble : isophane mixtures (Mixtard and Initard) gave similar results to our own mixtures of similar composition at equilibrium, i.e. Mixtard 3 : 7 chromatographically, is approximately 1 : 6 and Initard 1 : 1 is 1 : 2. However, the biological action of such mixtures upon injection is identical to that seen when the insulins are injected separately into different sites (Berger et al 1982), so these latter results would again seem to be only chemically, and not pharmacologically, relevant. Our in-vitro results agree with the in-vivo and in-vitro findings of Galloway et al (1982) and Nolte et al (1983), respectively.

In addition to possible interactions between different insulins, other potential sources of error have been recorded; these include cases of hypoglycaemic attacks in previously well controlled patients (Alexander 1984; Hall et al 1984). This has been attributed to 'dead' space in the syringes. This is the volume of a solution contained in the hub and needle when the plunger is fully depressed. There is no problem when only a single insulin is drawn into the syringe. However, drawing two types of insulin into a syringe with a large 'dead' space can lead to incorrect ratios being mixed. Hall et al (1984) measured the 'dead' spaces of glass and plastic syringes and found that the new BS1619 glass syringes (both 0·5 and 1·0 mL sizes) had a 'dead' space of 5 units. In contrast, new plastic disposable syringes had an insignificant 'dead' space. Our measurements showed a total volume of 1·004 ± 0·003 mL (n = 10) for the Plastipak disposable syringes used in our study. This is in close agreement with the figure quoted by Corcoran & Yudkin (1985) of 1·002 ± 0·002 mL. for the purposes of our work, therefore, the problem of 'dead' space was considered insignificant. However, if a BS1619 syringe with a 'dead' space equivalent to 5 units were used, the effects might be significant. From our results, the available soluble insulin will be reduced due to combination with free zinc, although not by as much as in a syringe with negligible 'dead' space. Thus, for a mixture of soluble and zinc insulin a large 'dead' space would be of slight benefit to the patient as it would lead to a higher level of soluble insulin being available after complexation than in a syringe with a negligible 'dead' space. The total amount of available soluble insulin would still however be much less than intended. For soluble and isophane mixtures, the clinical result is different. More soluble and less isophane insulin than originally intended would be available. Thus hypoglycaemic attacks are a real possibility when these insulins are mixed in glass syringes with a large 'dead' space. For plastic syringes, the negligible 'dead' space would help overcome this problem.

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