Biological potency of porcine, bovine and human insulins in the rabbit bioassay system

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Summary. Potency determination of porcine, bovine and human insulins relative to the International Standard in the pharmacopoeial rabbit bioassay system requires that the log-dose response curves are parallel. Furthermore, the same relative potency should be obtained independent of how the hypoglycaemic response is defined. The results of 508 rabbit blood glucose assays have been analyzed by new multivariate statistical methods. No deviations from parallelism of the log-dose response curves were detected. However, the potencies showed significant variation depending on the blood sampling times. Pure porcine and human (semisynthetic and biosynthetic) insulin potencies decreased by 12% and 18%, respectively, from the 30-min to the 2.5-h response, whereas bovine insulin potencies increased by 9%. Since the standard is a 52:48 mixture of bovine and porcine insulins, these results could be due to porcine and human insulins having a quicker onset and shorter duration of hypoglycaemic effect than bovine insulin. This was confirmed in assays of bovine relative to porcine insulin and by direct comparison of mean blood glucose curves. It is concluded that there is a response time-dependent variation in potency when the test and standard insulin have a different species composition. Hence, pure species insulin standards – a porcine, a bovine and a human standard – are needed for assay of the three insulins.

Key words: Bovine insulin, porcine insulin, human insulin, biological potency, rabbit bioassay, duration of hypoglycaemic effect, multivariate statistical analysis.

Differences in amino acid sequence between porcine, bovine and human insulin give rise to different physico-chemical properties [1–3] which are used in preparing pharmaceutical insulin preparations with different timing of action [4]. The different physico-chemical properties could cause a different hypoglycaemic effect in the pharmacopoeial bioassays which would render the results invalid if the test and standard insulin were of different species composition. A prerequisite for using the rabbit blood glucose assay for potency determination of bovine, porcine and human insulins relative to the present mixed bovine/porcine insulin standard is that the three insulin species exert similar hypoglycaemic effects in rabbits, i.e. the log-dose response curves must be parallel, and the same relative potency should be obtained independent of the response definition. However, an analysis of rabbit blood glucose assays by new multivariate statistical methods has shown that the relative potencies of porcine and bovine insulins based on blood glucose responses 30 min, 1 h and 2.5 h after the injection are significantly different [5].

In the present investigation results of 508 rabbit blood glucose assays of porcine, bovine and human insulins carried out in two different laboratories and in two different strains of rabbits are analysed by the multivariate statistical methods [6, 7] in order to determine how the potency of these insulins relative to the International mixed species Standard or to a pure pork insulin standard depends on the blood sampling time. The results of some of the previously analysed assays [5] are included in the extended and more detailed analyses given here.

Materials and methods

Insulins

A total of 74 insulin batches were examined, 72 of which were manufactured between 1972 and 1983 by Novo Industrie, Copenhagen, Denmark and used for pharmaceutical insulin preparations for the treatment of diabetes mellitus. The remaining two insulins were two batches of Humulin (Neutral Regular Human Insulin) made from biosynthethic human insulin produced by Eli Lilly & Co, Indianapolis, Indiana, USA. Fifteen batches recrystallized (RC) porcine insulin, 24 batches RC bovine insulin, 19 batches monocomponent (MC) porcine insulin, nine batches MC bovine insulin, five batches MC human insulin (semisynthetic) and two batches biosynthetic (BS) human insulin were studied.

Table 1. Content of various contaminants (pancreatic proteins and polypeptides) in the insulins as determined by radioimmunoassays: proinsulin-like substances [13], glucagon-like substances [14], pancreatic polypeptide [15], somatostatin [16] and vasoactive intestinal polypeptide [17]

Contaminants	MC insulins (porcine, bovine and human)	Recrystallized insulins		Fourth International Standard
		Porcine	Bovine	
Proinsulin-like substances	≤1	~15000	~25000	30000
Glucagon-like substances	≤0.1	~4	~10	700
Pancreatic poly-peptide	≤0.01	~3	~3	17
Somatostatin	≤0.01	~0.05	~0.07	0.1
Vasoactive intestinal polypeptide	≤0.01	~0.02	~0.03	0.5

All values are given in parts per million (ppm) by weight of the dry insulin

The porcine and bovine insulins were extracted from pork and beef pancreas glands, whereas the human insulins were made from either porcine insulin by enzymatic transpeptidation (semisynthetic human insulin) as described by Markussen [8] or by recombinant DNA technology (biosynthetic human insulin) as described by Chance et al. [9]. The RC insulins containing about 90% pure insulin were manufactured by conventional manufacturing processes without chromatographic purifications ending with four, and occasionally more, crystallizations of the insulin. The MC insulins were purified to more than 99% purity using a sequence of chromatographic purification processes including anion-exchange chromatography in an ethanolic medium [10–12].

Table 1 illustrates the purity of the RC and MC insulins by the content of pancreatic protein and polypeptide contaminants as determined by radioimmunoassays. For comparison, the content of these impurities in the Fourth International Standard for Insulin is shown as well. The human insulins, both those prepared by semisynthesis and purified to MC purity and those prepared by biosynthesis, are characterized by consisting of insulin, which in several chemical and physico-chemical tests behave identically with natural human insulin from human pancreas [9, 18]. The most convincing single identity test for the semisynthetic as well as the biosynthetic human insulin is the comparison of X-ray diffraction patterns of the insulin crystals, which proves identity of sequence, conformation and state of aggregation with that of natural human insulin [1]. The purity and identity of the insulins as determined by various analytical methods including HPLC is described in more detail [12, 18, 19] (RC & MC insulins); [9] (BS insulins).

Bioassays

The biological potency of the insulins was determined by the twin cross-over rabbit blood glucose assay procedure as described in the United States Pharmacopeia (USP) [20]. The majority of the bioassays were routine assays carried out in the Novo laboratory and the Food and Drug Administration laboratory (FDA) in connection with certification of batches of porcine, bovine and human insulins. The standard preparation was either the Fourth International Standard of Insulin – 52% bovine and 48% porcine insulin with a defined potency of 24.0 IU/mg [21] – or the USP Insulin Reference Standard G, which is identical to the International Standard. In addition, a number of bioassays of MC porcine, bovine and human insulin relative to MC porcine insulin were carried out.

For each assay, 28 (24) male or female rabbits of Rex White strain (Novo) weighing between 1.8 and 3.2 kg or New Zealand White strain (FDA & Novo) weighing between 2.6 and 5.0 kg were used in a randomized twin cross-over design. The rabbits were used for about 5 assays per year within a living period of 1–3 years (mean 2 years) in the laboratory. To secure that no insulin antibody formation, which could interfere with the bioassays, occurs in rabbits used this way, serum samples from a representative sample of 251 rabbits (approximately 20% of the rabbits used in this investigation) have been analyzed for insulin antibodies by adding ^{125}I-ox-insulin and determining the percent bound to antibody as described by Schlichtkrull et al. [12]. Only 13 of the serum samples showed more than 8% bound insulin, which is the detection limit of the analysis, and the highest measured value was 13%. The distribution of % bound was not different from that obtained on serum samples from rabbits never injected with insulin.

The rabbits were fasted 16–18 h before subcutaneous injection with dilutions of either the standard or the test preparation. Two dilutions of the standard and two dilutions of the test preparation containing 1.0 and 2.0 IU/ml, respectively, were prepared by diluting portions of stock solutions (40 IU/ml) with a 1.6% glycerol solution acidified with hydrochlorid acid to pH 3 and containing 0.1% phenol. The dilutions were stored at 4 °C and used within one week. The doses injected were either 0.40 and 0.80 IU/rabbit or 0.50 and 1.00 IU/rabbit with a period of 1 week (Novo) or one day (FDA) between the two injections. In some of the assays a blood sample was taken at 30 min after the injection in addition to the standardized 1 h and 2.5 h samples. In one set of assays samples were taken at 0.30 min, 1 h, 2.5 h and 4 h after the injection. All blood samples were 100 µl (Novo) or 800 µl (FDA) taken from the marginal ear vein. The blood glucose concentration was determined by autoanalyzer using either the ferricyanide method [22] or the hexokinase/glucose-6-phosphate dehydrogenase method [23]. The twin cross-over assay on 28 (24) rabbits was usually repeated four to six times and occasionally more, to obtain a potency estimate corresponding to a statistical weight of about 4000 or more. The statistical weight is defined as the reciprocal value of the variance of the $\log_{10}$ potency estimate, and the value of 4000 corresponds to a coefficient of variation of about 4%. A total of 508 single assays was carried out, corresponding to the use of about 14,000 rabbits.

Statistical analysis

The results of the assays were analysed according to standard statistical methods for twin cross-over assays [24] using as response the sum of the 1 h and 2.5 h blood glucose values as described in the pharmacopeia (USP) [20]. In addition, recently developed multivariate methods [6, 7] were used to analyse the responses at the various sampling times to investigate time dependent differences in potency between the insulin species. The results from each assay were analysed by univariate and multivariate analysis of variance to check the parallelism of the dose response curves for the test and standard. The potency estimates based on the sum of the 1 h and 2.5 h responses of the repeated assays of the same insulin batch were combined according to the weighted mean method [20] which includes a χ^2-test of homogeneity of the potencies. The multivariate potencies were combined according to maximum likelihood methods [7], which enable the testing of the homogeneity between responses (sampling times) and assays according to the two-way structure. First it was tested whether potency differences between responses and assays were additive (in log-potency); if they were, the differences between responses and assays could be tested, and combined potency estimates for each response could be calculated. Combined estimates for each response and batch were further combined to weighted mean potencies for batches of the same species and purity. Although there was statistically significant non-additivity in potency between assays and responses with a few batches, none were excluded, and the homogeneity of the potencies across batches was checked by means of χ^2-tests. All the statistical analyses were carried out by means of specially developed APL computer programs.

Table 2. Recrystallized porcine (RCP) and bovine (RCB) insulin batches relative to the International Standard

| Batch No. | Laboratory | No. of assays | Biological potency estimates with ±95% confidence limits (IU/mg nitrogen) | | | Hetero-geneity | BG [1 h + 2.5 h] |
| | | | Blood glucose response | | | | |
			BG 30 min	BG 1 h	BG 2.5 h		
RCP1	Novo	4		178	174		173 ± 17
RCP2	Novo	3		194	166	T	174 ± 18
RCP3	Novo	8		198	173		179 ± 12
RCP4	Novo	7		187	180		182 ± 11
RCP5	Novo	3		186	164		169 ± 17
RCP6	Novo	4		175	168		170 ± 15
RCP7	Novo	4		186	159	T	169 ± 12
RCP8	FDA	7		182	172		174 ± 12
RCP8	Novo	4		159	157	A*T	160 ± 14
RCP9	FDA	5		183	168	A + T	171 ± 12
RCP9	Novo	5		162	180	A + T	177 ± 16
RCP10	FDA	6		186	175		180 ± 14
RCP10	Novo	4		175	165		171 ± 16
RCP11	FDA	4		168	159	A	164 ± 11
RCP11	Novo	4	177	198	173	T	178 ± 15
RCP12	FDA	6		180	175	A*T	170 ± 9
RCP12	Novo	8	173	176	162		168 ± 10
RCP13	FDA	6		178	176		175 ± 14
RCP13	Novo	4		177	165		170 ± 12
RCP14	FDA	5		179	169	A*T	172 ± 12
RCP14	Novo	3		182	175		177 ± 15
RCP15	FDA	5		176	168		173 ± 15
RCP15	Novo	4		201	164	A*T	178 ± 15
RCB1	Novo	4		179	178		178 ± 12
RCB2	Novo	5		181	177	A*T	176 ± 14
RCB3	Novo	6		172	182		179 ± 14
RCB4	Novo	4		170	181		179 ± 13
RCB5	FDA	7		155	166		163 ± 12
RCB5	Novo	6		155	172	A + T	169 ± 12
RCB6	FDA	6		178	188		185 ± 14
RCB6	Novo	4		162	172	A	169 ± 13
RCB7	FDA	4		163	173		169 ± 12
RCB7	Novo	6		177	174		175 ± 14
RCB8	FDA	6		173	176		174 ± 14
RCB8	Novo	5		166	168	A*T	167 ± 13
RCB9	Novo	5		145	174	A*T	167 ± 11
RCB10	FDA	5		165	178	T	172 ± 13
RCB10	Novo	5		156	178	A*T	171 ± 12
RCB11	FDA	5		175	178	A	180 ± 12
RCB11	Novo	4		151	200	A + T	181 ± 15
RCB12	FDA	6		182	185		183 ± 14
RCB12	Novo	4		167	184	T	176 ± 14
RCB13	FDA	4		165	173		171 ± 16
RCB13	Novo	4		172	174		174 ± 16
RCB14	FDA	5		171	169		171 ± 12
RCB14	Novo	6		160	181	A + T	173 ± 10
RCB15	FDA	7		170	173		172 ± 12
RCB15	Novo	3		181	186		186 ± 14
RCB16	FDA	5		157	174		168 ± 15
RCB16	Novo	3		161	191	T	180 ± 15
RCB17	FDA	5		174	182		177 ± 14
RCB17	Novo	6		160	176		171 ± 13
RCB18	FDA	5		182	163	T	169 ± 14
RCB18	Novo	4		188	182		183 ± 16
RCB19	FDA	4		178	175		181 ± 15
RCB19	Novo	4		173	179		178 ± 13
RCB20	FDA	5		169	180		175 ± 14
RCB20	Novo	4		186	183		182 ± 14
RCB21	FDA	6		178	173		178 ± 13
RCB21	Novo	4		165	174		173 ± 14
RCB22	FDA	5		168	174		173 ± 13
RCB22	Novo	4		184	190		188 ± 15
RCB23	FDA	4		169	182		176 ± 15
RCB23	Novo	4		169	176		174 ± 13
RCB24	FDA	6		164	171	A	170 ± 14
RCB24	Novo	4		176	185		182 ± 13

Statistically significant heterogeneity in potency ($p < 0.05$) is indicated as follows: A + T: assays and sampling times (additive effects on log-potency), A*T: assays and sampling times (non-additive effects on log-potency), A: assays only, T: sampling times only

Table 3. MC porcine, MC bovine, and MC and BS human insulin batches relative to the International Standard

Batch no.	Laboratory	No. of assays	Biological potency estimates with ±95% confidence limits (IU/mg nitrogen or %)			Hetero-geneity	BG [1 h + 2.5 h]
			Blood glucose response				
			BG 30 min	BG 1 h	BG 2.5 h		
MCP1	Novo	4		177	172	A	174±13
MCP2	Novo	4		175	163	A+T	170±14
MCP3	Novo	6		179	159	T	167±13
MCP4	Novo	6	211	175	157	T	163±15
MCP5	Novo	6	200	180	168		174±16
MCP6	FDA	5		194	183		185±12
MCP6	Novo	6	194	183	179	A*T	178±12
MCP7	FDA	5		197	164	A+T	175±13
MCP7	Novo	4	204	185	162	A+T	170±15
MCP8	FDA	5		198	177	T	187±14
MCP8	Novo	6	196	186	161	A+T	166±10
MCP9	Novo	6	173	177	171		171±10
MCP10	Novo	5		198	169	T	179±16
MCP11	Novo	3		194	185	A	189±14
MCB1	Novo	6		173	182		179±13
MCB2	Novo	4		187	195		194±16
MCB3	Novo	4		189	189		189±13
MCB4	Novo	4		160	185	A	178±16
MCB5	FDA	6		185	195		191±16
MCB5	Novo	11	159	162	183	T	177±10
MCB6	FDA	5		175	186		183±14
MCB6	Novo	4	177	182	182		181±14
MCH1	Novo	4	247	191	178	A+T	184±12
MCH2	Novo	4	208	187	164	T	175±15
MCH3	Novo	8		182	166	T	173± 9
MCH4	Novo	6	205	170	157	T	166±10
MCH5	Novo	3	196	177	178		179±14
BSH1	Novo	3	107%	109%	99%		102± 6%
BSH2	Novo	4	112%	95%	89%	T	91± 5%

Statistically significant heterogeneity in potency ($p < 0.05$) is indicated as follows: A+T: assays and sampling times (additive effects on log-potency), A*T: assays and sampling times (non-additive effects on log-potency), A: assays only, T: sampling times only

Results

Porcine, bovine and human insulin, International Standard

The results of 468 single rabbit assays on porcine (RC and MC), bovine (RC and MC) and human (MC and BS) insulin relative to the International Standard are shown in Tables 2 and 3. Of these, 298 assays were carried out in the Novo laboratory and 170 in the FDA laboratory. The potency estimates are determined from the blood glucose responses 1 h and 2.5 h after the injection, plus, in some cases, also after 30 min, and are expressed in either IU/mg nitrogen or percent of labelled potency. The columns to the right show the univariate potencies with 95% confidence limits calculated by standard methods for twin cross-over assays using as response the sum of the 1 h and 2.5 h blood glucose values [20, 24]. The remaining potencies corresponding to the 30-min, 1-h, and 2.5-h responses are obtained by the multivariate analysis with tests for heterogeneity [6, 7].

By the multivariate and univariate statistical analyses no significant deviations from parallelism of the log-dose response curves could be detected in these assays. The tests of homogeneity that were carried out in connection with the combination of results of single assays on the same batch showed, however, significant ($p < 0.05$) heterogeneity to be present in 32 out of the 63 batches assayed at Novo and in 10 out of the 32 batches assayed at FDA.

Combined (weighted mean) potencies of porcine, bovine and human insulins from Tables 2 and 3, together with the underlying potencies of each batch, are shown in Figures 1 and 2. The combined potencies of MC porcine and MC and BS human insulins decrease by 4% and 9%, respectively, from the 30 min to the 1 h response and further by 8% and 9% from the 1 h to the 2.5 h response. The combined potency of MC bovine insulin increases by 3% from the 30 min to the 1 h response and further by 6% from the 1 h to the 2.5 h response. Similar but somewhat smaller potency differences are present in assays of RC porcine and bovine insulins. The decrease and increase in combined potencies (weighted means from both laboratories) for these insulins from the 1 h to the 2.5 h response are 6% and 4%, respectively. The smaller potency differences in as-

Table 4. MC porcine, MC bovine and MC human insulin batches relative to MC porcine insulin batches

Batch no.	Batch no.	No. of assays	Relative potency estimates with ±95% confidence limits (%)			Hetero-geneity	BG [1 h + 2.5 h]
			Blood glucose response				
			BG 30 min	BG 1 h	BG 2.5 h		
MCP12	MCP13	2	80.5	95.4	104.6		101 ± 11
MCP8	MCP14	4	103.1	104.5	97.2		99 ± 8
MCP8	MCP15	4	111.0	101.1	105.9		104 ± 8
MCB1	MCP16	4	81.9	99.8	116.1	T	111 ± 11
MCB7	MCP17	4		96.8	108.6	T	104 ± 9
MCB4	MCP2	4		95.4	108.3	T	102 ± 9
MCB8	MCP4	6	108.2	107.8	103.8	A	105 ± 8
MCB5	MCP6	4	82.5	96.3	107.8	A*T	103 ± 8
MCB9	MCP18	4	92.3	90.4	108.8	A*T	103 ± 10
MCH1	MCP19	4	96.7	103.5	103.3	A	102 ± 6

Statistically significant heterogeneity in potency (*p* < 0.05) is indicated as follows: A*T: assays and sampling times (non-additive effects on log-potency), A: assays only, T: sampling times only
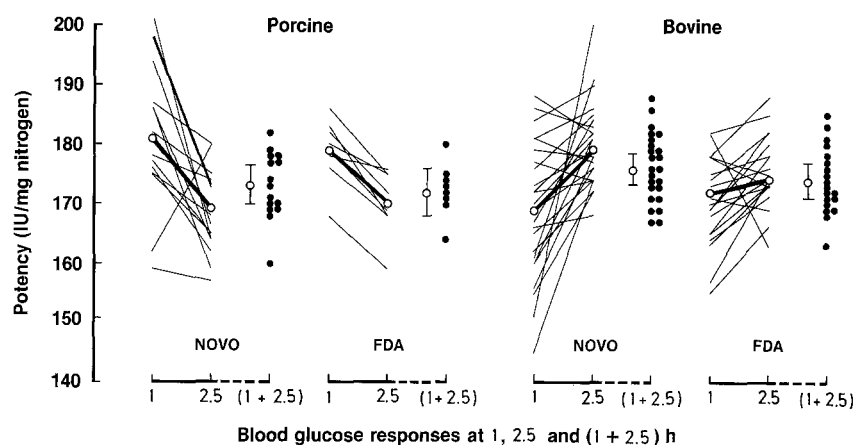
Fig. 1. Combined (weighted mean) multivariate potency based on blood glucose responses at 1 h and 2.5 h and combined (weighted mean) univariate (USP method) potency based on the sum of the 1-h and 2.5-h responses for 15 batches of RC porcine and 24 batches of RC bovine insulin relative to the International Standard (O—O). The vertical bars indicate 95% confidence limits. The underlying potencies of each batch are connected with thin lines (multivariate potencies) and shown as points (univariate potencies)

says of RC insulins can be explained by the presence of impurities with a timing of action different from that of insulin. The RC insulins as well as the standard contain, for instance, 2–3% proinsulin, proinsulin-intermediates and other proinsulin-like substances (Table 1) which have a prolonged hypoglycaemic effect compared to insulin.

Mean blood glucose curves from six assays of one batch of human insulin relative to the International Standard (MCH4 in Table 3) are shown in Figure 3.

MC porcine, bovine and human insulin/MC porcine insulin

The results of 40 single rabbit assays all carried out in the Novo laboratory on MC porcine, bovine and human insulin relative to MC porcine insulin are shown in Table 4. The potencies are determined from the blood glucose responses 1 h and 2.5 h after the injection plus, in some cases, also after 30 min, and are expressed in percent of the molar potency (168 · 10⁶ IU/mol insulin ~ 184 IU/mg nitrogen) [25]. The columns to the right show the univariate potencies with 95% confidence lim-

its calculated by standard methods for twin cross-over assays using as response the sum of the 1 h and 2.5 h blood glucose values [20, 24]. The remaining potencies, corresponding to the 30 min, 1 h, and 2.5 h responses, are obtained by the multivariate analysis with tests for heterogeneity [6, 7].

By the multivariate and univariate statistical analyses, no significant deviations from parallelism of the log-dose response curves could be detected in these assays. The tests of homogeneity that were carried out in connection with the combination of results of single assays on the same batch showed, however, significant (*p* < 0.05) heterogeneity to be present in all assay combinations where the test and standard were of different species, whereas no assay combinations where porcine insulin was assayed relative to porcine insulin showed significant heterogeneity.

Combined (weighted mean) potencies of the MCB/MCP assays from Table 4 together with the underlying potencies of each batch are shown in Figure 4. The combined potency of MC bovine relative to MC porcine insulin increase by 3% from the 30 min to the 1 h response and further by 11% from the 1 h to the 2.5 h re-
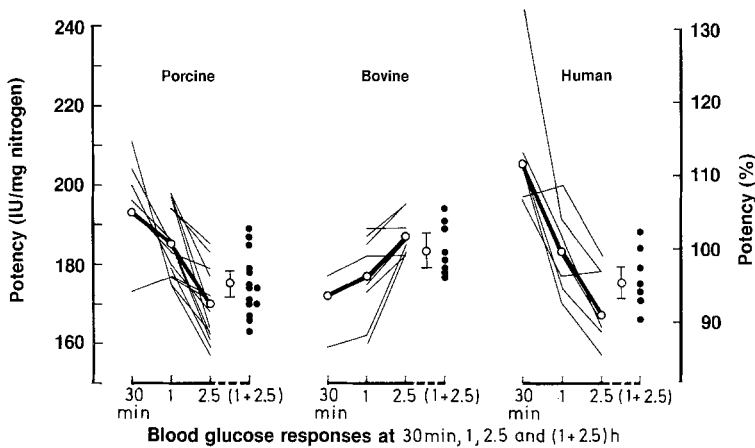
Fig. 2. Combined (weighted mean) multivariate potency based on blood glucose responses at 30 min, 1 h and 2.5 h and combined (weighted mean) univariate (USP method) potency based on the sum of the 1-h and 2.5-h responses for 11 batches of MC porcine, 6 batches of MC bovine, 5 batches of MC human and 2 batches of BS human insulin relative to the International Standard O—O. The vertical bars indicate 95% confidence limits. The underlying potencies of each batch are connected with thin lines (multivariate potencies) and shown as points (univariate potencies)
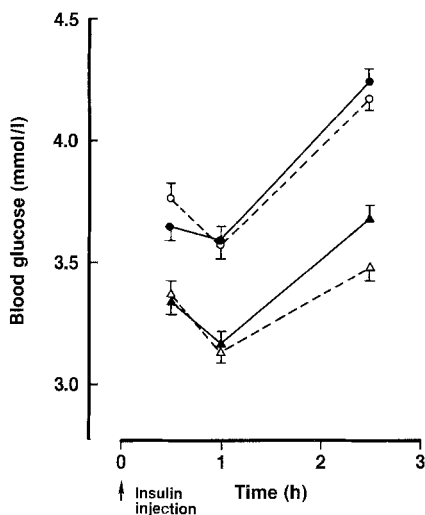
Fig. 3. Blood glucose curves from six repeated assays (168 rabbits) of a batch of MC human insulin relative to the International Standard. The dose injected was either 0.5 IU/rabbit (human ●—●, Standard O-----O) or 1.0 IU/rabbit (human ▲—▲, Standard △-----△). Each point and interval represents the mean ± SEM

sponse. Mean blood glucose curves from four assays of one batch of bovine relative to one batch of porcine insulin (MCB1/MCP16 in Table 4) are shown in Figure 5.

Discussion

The results indicate a systematic response time-dependent variation in potency in the rabbit bioassay when the test and standard are of different species composition. For instance, 36 out of 37 assays of porcine insulin and 6 out of 7 assays of human insulin relative to the mixed species standard show a higher potency estimate with the 1-h response than with the 2.5-h response, whereas 40 out of 51 assays of bovine insulin show a lower potency estimate with the 1-h response than with the 2.5-h response (Tables 2 and 3). The 30-min response gives an even higher/lower potency estimate. Since the standard is a 52:48 mixture of bovine and

porcine insulin, these results could be due to porcine and human insulin having a quicker onset and shorter duration of hypoglycaemic effect in rabbits than bovine insulin. That porcine insulin in fact has a quicker and less prolonged hypoglycaemic effect is shown directly in Figure 5, where mean blood glucose curves of bovine relative to porcine insulin are compared. Figure 3 illustrates in a similar way that human insulin has a quicker and less prolonged hypoglycaemic effect compared with the mixed bovine/porcine insulin standard. The data in Table 3 and Figure 2 indicate that human insulin may have an even quicker onset than porcine insulin.

The systematic response time-dependent variation in potency is the same, no matter which laboratory/rabbits have been used in the bioassays. The potency of porcine, bovine and human insulin relative to the present International mixed species Standard cannot be expressed by a single number, since potency varies with the blood sampling time. This invalidity is due to differences between the standard and the unknown, as the assay per definition must be valid when these are identical. In the pharmacopoeial bioassay design (response: blood glucose [1 + 2.5] h) using the International Standard as the standard the response time-dependent variation in potency results in an, on the average, 5% underestimation of the potency of MC porcine and MC and BS human insulin (175 IU/mg nitrogen instead of 184 IU/mg nitrogen), whereas MC bovine insulin accidentally gives an estimate very close to the molar potency of these pure insulins ($168 \cdot 10^6$ IU/mol insulin ~ 184 IU/mg nitrogen) [25]. One could suggest that, by selection of a suitable blood sampling time or by designing a special combination of the responses measured at different times, it might be possible to make the rabbit blood glucose assay insensitive to the differences between the three insulin species. Considering the biological variation in the blood glucose curves, such an approach cannot be expected to solve the problem in general. The use of an insulin standard of the same species or species mixture as the unknown will, however, secure that the assay gives valid results.
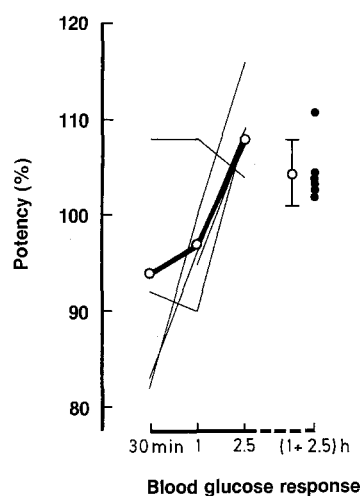
Blood glucose response

Fig. 4. Combined (weighted mean) multivariate potency based on blood glucose responses at 30 min, 1 h and 2.5 h and combined (weighted mean) univariate (USP method) potency based on the sum of the 1-h- and 2.5-h responses for six batches of MC bovine insulin, using six batches of MC porcine insulin as standard (26 assays in all) O——O. The vertical bars indicate 95% confidence limits. The underlying potencies of each batch are connected with thin lines (multivariate potencies) and shown as points (univariate potencies)
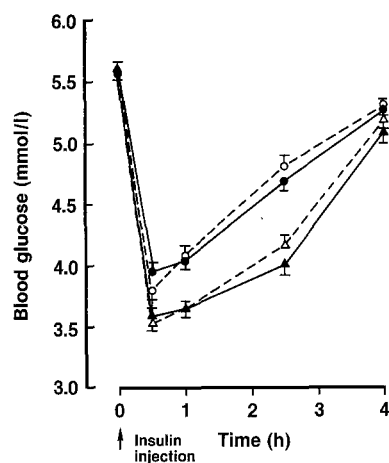
Fig. 5. Blood glucose curves from four repeated assays (112 rabbits) of a batch of MC bovine insulin relative to a batch of MC porcine insulin. The dose injected was either 0.5 IU/rabbit (bovine ●——●, porcine O-----O) or 1.0 IU/rabbit (bovine ▲——▲, porcine △-----△). Each point and interval represents the mean ± SEM

In this connection it should be mentioned that there is no evidence of any problem with the other officially accepted pharmacopoeial bioassay systems – the mouse convulsion and the mouse blood glucose assay – concerning differences in the timing of the hypoglycaemic effect of various insulin species, and that no statistically significant differences between mean biological potencies of MC porcine, bovine and human insulins have been found in the mouse convulsion assay [25].

In addition to the systematic response time-dependent variation in potency, the results in the right column of Table 3 illustrate the random variation in potency es-

timates of the pure and uniform MC insulins (> 99% insulin), which is solely due to variations in the assay. To avoid the inevitable batch-to-batch variation in the final insulin preparations, if the potency is assessed based on a bioassay, the potency of the pure insulins should be defined on a molar basis and assessed based on accurate chemical analysis [25, 26]. Bioassays should be performed in addition to various modern analytical methods, including the RIA and HPLC, to ensure identity and purity of the insulins. This is in accordance with the recommendation given by Home and Alberti [27] in their review of human insulin, "what is required for studies in humans is a comparison of equimolar amounts of human and pork insulin, not equivalent activities in mouse or rabbit".

Clinical studies of human and porcine insulin have shown a tendency to a more rapid onset of hypoglycaemic effect with the human insulin – both semisynthetic and biosynthetic – when neutral soluble preparations are injected subcutaneously into normal subjects [28-30]. In some studies a stronger overall hypoglycaemic effect of the human insulin has been observed [31-34]. Pharmacokinetic studies have indicated that neutral soluble human insulin tends to be more quickly absorbed after subcutaneous injection than the corresponding porcine insulin [28, 33-37]. In a recent study where neutral solutions of human, porcine and bovine insulin were injected subcutaneously into normal subjects, significant differences in hypoglycaemic effect – similar to those seen in the rabbit bioassay – have been found [38].

In conclusion, porcine and human insulin have a quicker onset and shorter duration of hypoglycaemic effect in rabbits than bovine insulin. Human insulin may have an even quicker onset than porcine insulin similar to the tendency seen in some clinical studies. In the rabbit bioassay this results in a response time-dependent variation in potency when the test and standard insulin have a different species composition. Hence, pure species insulin standards – a porcine, a bovine and a human standard – are needed for assays of porcine, bovine and human insulin in this bioassay system.

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