Adsorption of Insulin to Infusion Bottles and Tubing

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SUMMARY

The adsorption of clinically used quantities of insulin to infusion systems was studied. Isotonic saline solutions containing tracer quantities of beef insulin-I-131, with or without varying amounts of nonradioiodinated (carrier) regular beef insulin, were added to bottles of 500 ml. infusion sets. The solutions were then allowed to flow out into collection beakers. Adsorption was assessed by measuring the decrease of radioactivity in the solutions and/or recovery from the bottles by 30 per cent potassium hydroxide (KOH). The influence of human serum albumin (HSA) on adsorption was evaluated by repeating the experiments after HSA was added to the infusion fluid.

Adsorption of insulin-I-131 to both bottles and tubing was considerable, especially at low concentrations of carrier insulin. HSA effectively reduced the adsorption to clinically insignificant amounts. DIABETES *17*:766-71, December, 1968.

Adsorption of insulin-I-131 from dilute solutions to laboratory glassware was first reported in 1951.[1] This observation has been confirmed[2-5] and large proportions of noniodinated insulin have also been found to be adsorbed to glass.[6] Newerly and Berson[2] noted decreasing but variable adsorption of insulin to glassware at concentrations from 0.09-500.0 ug./ml., and Freinkel and Goodner[4] reported as much as 46.7 per cent loss in small beakers at a concentration of 0.25 ug./ml. This binding is considered a nonspecific surface phenomenon which may occur on other inert materials such as paper,[2] polyethylene[3] and siliconized and pyrex glassware.[3]

No data are available on insulin loss by adsorption when it is administered by intravenous infusion for therapeutic purposes. Since the insulin concentrations in infusions generally are in the range in which adsorption has been reported, it was considered worthwhile to investigate the amount of insulin lost to the glassware and tubing in such systems.

MATERIAL AND METHODS

The general experimental procedure was to add isotonic saline solutions containing known tracer quantities of radioiodinated beef insulin,* with or without known varied amounts of nonradioiodinated (carrier) regular beef insulin,† to the bottles of conventional 500 ml. infusion sets.‡ These solutions then were allowed to empty into collection beakers. Adsorption to the glass bottles and/or plastic tubing was assessed by (a) comparing the radioactivity present in samples obtained at various time intervals from the bottles and/or the collection beakers with that of the initial solution, and (b) measuring the amount of radioactivity that could be recovered by 30 per cent potassium hydroxide (KOH) rinses[5] of the bottles. Each infusion set was used once. Radioactivity was measured by placing aliquots of the solutions in small vials and counting them in a well-type scintillation detector attached to a conventional scaler. Final tracer insulin concentrations varied between 2.6×10^{-4} and 8.0×10^{-2} ug./ml. Final carrier insulin concentrations varied between 0.16 and 3.2 ug./ml. (2-40 U. per 500 ml.).

Preliminary experiments indicated that NaI-131 was not significantly adsorbed to glassware. Any small amount remaining in an emptied bottle could be easily

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*Beef insulin-I-131 was obtained from Abbott Laboratories or was iodinated in our laboratory by the method of Hunter and Greenwood,[10] and purified prior to use so that it contained less than 5 per cent damaged material.

†Squibb beef crystalline zinc insulin (U-40), containing 1.6 mg./ml. or 1.0 U./0.04 mg.

‡Abbott Laboratories Abbo-liter Containers (glass) and Venopak or Venopak Micro-drip Venoclysis Sets (polyvinyl chloride tubing).

removed by water rinses; KOH rinsing did not remove more. In contrast, water or saline washes removed only small amounts of insulin-I-131 left in a bottle (presumably a superficial "wet layer" remaining behind), whereas a high percentage of remaining counts could be recovered by 30 per cent KOH rinses, even after first rinsing with water.

RESULTS

1. *Adsorption to infusion bottle*

Varying amounts of carrier insulin were mixed with 10 ml. of a tracer insulin solution in screw-capped glass vials for one minute. The contents and then a saline rinse were emptied into 500 ml. infusion bottles filled with isotonic saline. The contents of the infusion bottles were then mixed manually by shaking for one minute and aliquots taken for counting (initial radioactivity). The liquid then flowed from the bottles through connected tubing sets at a rate so that the bottles emptied in two hours. The bottles were drained of any remaining fluid and 75 ml. of 30 per cent KOH added. The bottles were then mechanically shaken, ninety times per minute, for thirty minutes. Aliquots of the KOH were taken and counted to calculate the total eluted radioactivity.

Figure 1 illustrates the relatively large but variable amount of insulin (19-33 per cent) that remains on the glassware at tracer levels and that this decreases with increasing concentrations of insulin.

2. *Adsorption to infusion bottle and tubing*

Similar experiments were performed except that the solution was allowed to run out more slowly into col-

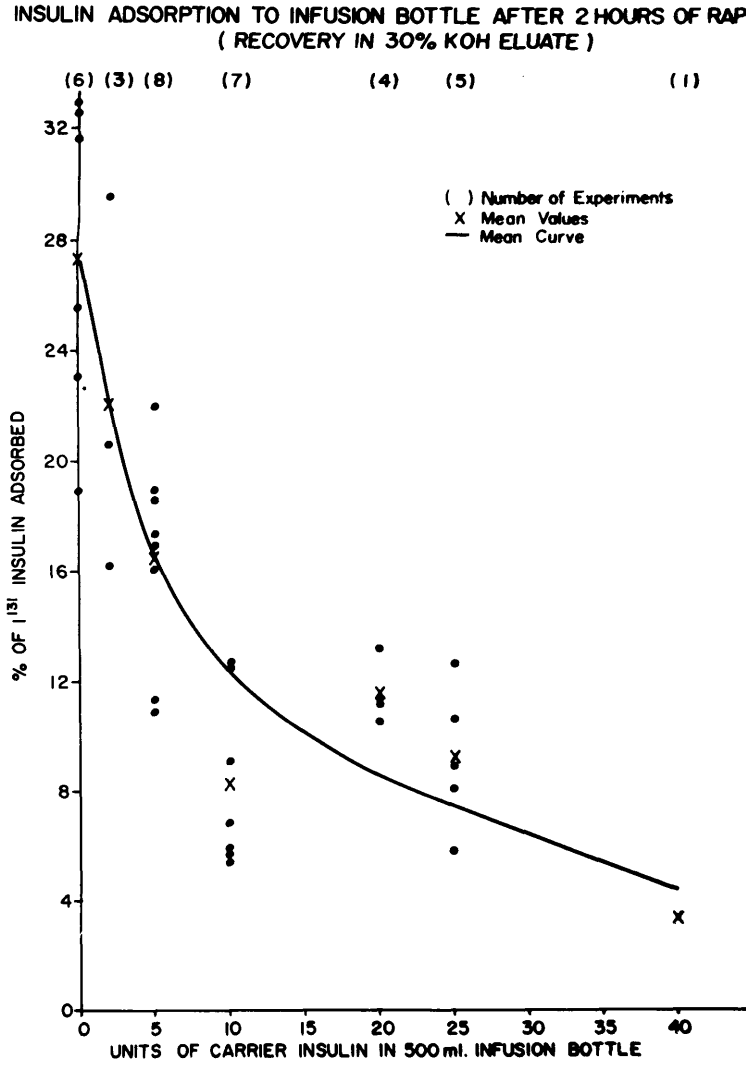

FIG. 1. The dots represent the per cent of initial insulin-I-131 recovered by KOH from individual infusion bottles after a two-hour emptying period.

lection beakers, at a rate of 120-150 ml. in two hours. The beakers were emptied at one hour and aliquots taken at two hours from the fluid collected in the second hour. Samples from fluid still in the infusion bottles were also taken at two hours. The bottles were then emptied, drained and eluted with 30 per cent KOH as described above. Samples of the eluates as well as the other samples were counted for radioactivity.

Figure 2 illustrates glassware and tubing adsorption of insulin after two hours. "A" represents the loss of radioactivity from the liquid still in the bottles after two hours. "B" represents the recovery of radioactivity from these bottles using KOH. "C" demonstrates the loss of activity from the liquid collected from the infusion setup at two hours, i.e., the loss due to adsorption by glassware plus tubing. All calculations were made using the radioactivity measured after one minute in the 500 ml. bottles as the 100 per cent figure.

As noted in figure 2, per cent adsorption decreases as the amount of insulin increases. The tubing accounts for a large portion of the insulin adsorbed. This is reflected in the difference between curves C and A or between C and B. The discrepancy between curves A and B is explained by the results of the next experiment. The quantitative difference between figure 1 and curve B of figure 2 may indicate that rapid flow is associated with greater glassware adsorption.

3. *Adsorption to infusion system with albumin added*

These experiments were performed in order to evaluate the effect of albumin on insulin adsorption. Tracer

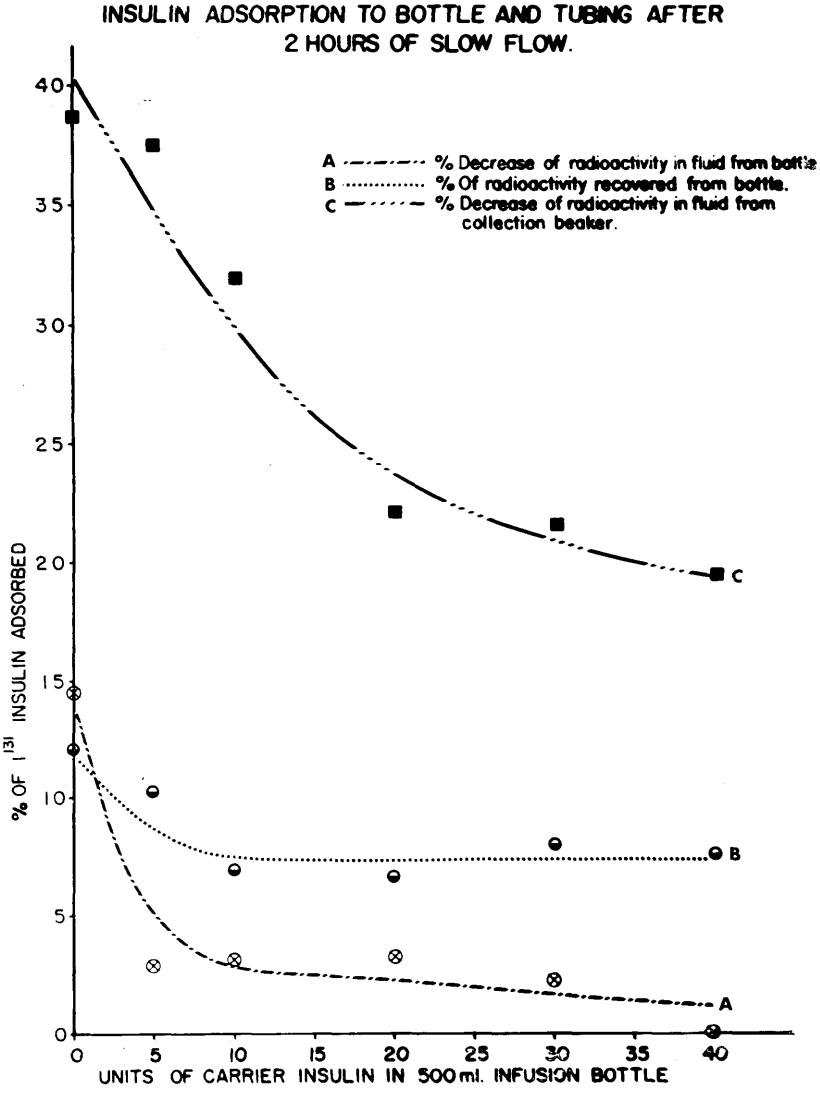

FIG. 2. Curves A, B and C are derived from the same experiments. Each point is the mean of ten observations. Curves A and B represent insulin adsorption to the infusion bottles as measured in two different ways. The difference between Curve A and C or B and C represents tubing adsorption.

solutions containing varying quantities of Regular Insulin were first mixed in large flasks and then poured into 500 ml. infusion systems. "Zero" aliquots were taken from the mixing flasks, infusion bottles and collection beakers. Thereafter infusions were run at a constant rate so that the bottles emptied in about two hours. Every thirty minutes each infusion was stopped, the tubing separated for a bottle sample to be removed, and then restarted to collect a fresh system (tubing) sample from the collection beaker. The experiments were repeated with the addition of pooled human serum albumin (HSA)* in a final concentration of 0.35 per cent. HSA is commonly used to prevent insulin adsorption to laboratory glassware.[4,5,7]

One experiment was done to determine the total loss of radioactivity, i.e., insulin adsorption, during a complete two-hour infusion and to evaluate adsorption to the collection beaker as a possible source of error in the previous data. Tracer iodoinsulin and 40 U. of Regular Insulin in 500 ml. saline were allowed to drip at a constant rate so that all the fluid emptied into a collection beaker in two hours. The beaker had previously been rinsed with 0.35 per cent HSA to prevent insulin adsorption. Aliquots from the total two-hour collection in the beaker were taken to determine the absolute amount of insulin a patient would receive in a typical clinical situation, as compared to the insulin dose added to the infusion bottle.

Tables 1 and 2 show that per cent of insulin lost by adsorption to infusion systems not containing HSA decreased as concentration increased, although loss at 40 U./bottle was still significant. Glass bottles and plastic tubing adsorbed the hormone, and both showed changes in per cent adsorption as the infusion proceeded (table 1).

By considering the count rate in the sample from the mixing flask as the 100 per cent level, it was demonstrated that there was an initial fall in radioactivity immediately after the insulin solution was added to the infusion bottle ("zero" bottle sample). In the bottle

*Hyland salt poor pooled human serum albumin, 25 per cent.

TABLE 1

Per cent adsorption of insulin at various time intervals during 500 ml. infusion

Source and time of specimen (min.)	NaI-131	Ins.-I-131 tracer	Tracer +5 U.	Tracer +10 U.	Tracer +20 U.	Tracer +40 U.	Tracer +5U. +HSA	Tracer +10 U. +HSA	Tracer +20 U. +HSA	Tracer +40 U. +HSA
Bottle "Zero"	2.6	14.1	10.5	9.2	5.0	3.1	0.7	0.1	0.5	2.0
Bottle 30	2.8	16.3	13.6	9.3	7.7	4.9	0.8	0.9	0.8	1.9
Bottle 60		16.8	16.3	10.2	9.9	6.4	2.6	0.3	0.2	1.5
Bottle 90	2.7	21.2	18.9	12.4	11.6	7.9	2.7	1.6	0.7	1.9
Bottle 120		26.1	21.2	13.3	11.5	8.0	2.0	0.8	0.6	1.8
Tubing "Zero"	3.1	46.4	37.9	31.5	30.0	26.2	6.5	5.2	2.8	7.6
Tubing 30	3.9	44.4	36.0	29.1	26.9	26.1	4.5	1.8	1.2	7.1
Tubing 60		43.3	33.1	28.0	25.4	18.5	5.3	3.2	1.0	4.3
Tubing 90	3.3	35.2	31.3	27.4	21.7	15.9	5.2	3.9	2.6	6.5
Tubing 120		34.5	30.5	25.0	20.4	14.1	4.4	4.4	1.4	6.6
No. of runs	1	4	2	2	2	2	2	2	2	2

TABLE 2

Adsorption of insulin to bottles and tubing at two hours

No. of Runs	Insulin concentration in ug./ml. (or units per bottle)	HSA concentration (mg./ml.)	Per cent decrease of initial radioactivity in system sample
4	Tracer (0)	0	34.5
2	0.4 (5)	0	30.5
2	0.8 (10)	0	25.0
2	1.6 (20)	0	20.4
2	3.2 (40)	0	14.1
1	3.2 (40)	0	16.0*
2	0.4 (5)	3.5	4.4
2	0.8 (10)	3.5	4.4
2	1.6 (20)	3.5	1.4
2	3.2 (40)	3.5	6.6

*Total loss over two-hour infusion period.

samples taken thereafter, continued loss of radioactivity became apparent although the increment at each time interval was much smaller than the initial adsorption. Increased adsorption to the bottles continued until the end of the infusion at two hours.

The immediate adsorption explains the discrepancy between curves A and B in figure 2 of the previous experiment. The 100 per cent level used did not take this loss into account. This would make curve A show less adsorption and curve B more adsorption than actually occurred.

Separate samples to determine the loss to the entire system showed a great drop in radioactivity (26.2 per cent at 40 U./bottle and 46.4 per cent at tracer-only concentration) in the first few milliliters which emerged from the tubing ("zero" tubing sample). Actual tubing adsorption could be determined by subtracting bottle loss from system loss. At each successive interval during the infusion there was a slightly smaller loss to the total system. When the increasing loss to the bottle is accounted for, this implies an initial large binding of insulin to the tubing, with decreased binding or increased elution as the solution continues to run through the tubing.

The presence of 0.35 per cent HSA almost completely prevented adsorption of insulin to the bottles and tubing loss was also markedly decreased.

In the one experiment in which this was evaluated, using 40 U. insulin/500 ml., the average total loss from all the fluid collected over a two-hour period (16.0 per cent) was similar to that found in freshly collected fluid obtained at two hours (14.1 per cent, table 2). This implies that a patient would receive approximately 34 of 40 U. of insulin added to a 500 ml. infusion system and that the total insulin loss at other concentrations is probably close to the percentage decrease at two hours as given in table 2. It also implies that rinsing the collection beakers with HSA, as done in this single experiment, would not have altered the results in the previous experiments. No attempt had been made to eliminate adsorption to collection beakers and pipettes, since it was considered the adsorption would be small relative to that of the infusion system and that any error would be a fairly uniform one.

DISCUSSION

From the data it is apparent that there is early and considerable insulin loss to infusion systems with doses that might be used clinically. We have confirmed the insulin adsorption to glassware frequently observed in laboratory apparatus,[1-6] and have demonstrated similar loss in tubing used for intravenous infusions.

It is apparent from the results that the magnitude of insulin that adsorbs to glass or tubing surfaces varies with many factors including: (1) concentration of insulin; (2) duration of time in contact with adsorbing surface; (3) rate of flow of insulin solution; and (4) presence of other negatively charged proteins such as HSA. Data from several small ancillary experiments, not reported here, confirm the impressions: (a) that turbulence, as in shaking or rapid flow, causes more glassware adsorption than when the fluid is motionless in or flows slowly from the container; (b) that the opposite is true of adsorption to tubing.

Insulin adsorption to laboratory glassware is greatly decreased when varying amounts of HSA (especially larger than 2 mg./ml.) are added.[4,5,7] We elected to use 0.35 per cent HSA (3.5 mg./ml.) because this is the concentration routinely used by our laboratory to prevent such insulin loss during immunoassays. In all the present experiments there was strikingly diminished loss of radioactivity to the infusion bottles and tubing.

Insulin in intravenous infusions has been effectively used to treat patients,[8,9] but such use may be considered an inefficient and inaccurate mode of administration. While the therapeutic significance of insulin adsorption to the bottle and tubing will depend on each clinical situation, the physician should be aware of its occurrence. Our data indicate that adding a small quantity of sterile human albumin, such as 7 ml. 25 per cent HSA to 500 ml. isotonic saline, will effectively prevent this loss of insulin. Such addition of HSA probably carries little risk and might be considered when it is the judgment of the physician to administer insulin to patients by infusion. Administration of insulin from a syringe directly into a vein or into tubing near a vein does not result in any significant insulin loss, since the solution is a concentrated one.

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Mental Development Following Nutritional Marasmus

...A study of a small group of children in Peru recovering from nutritional marasmus is of special interest (E. Pollitt and G. Granoff, Rev. Interam. Psicologia *1*:93, 1967). The nineteen study subjects were children who had been admitted to hospital primarily because of severe malnutrition. At the time of admission none had edema, eighteen had normal serum albumin levels, and all were below 60 per cent of their expected weight for age. They were diagnosed as suffering from nutritional marasmus and received treatment in hospital. At the time of their mental evaluation all were still under thirty-six months of age and had clinically recovered from their severe marasmus. Eight control children who were siblings of the study subjects were enrolled for testing. They all satisfied certain selective criteria, including having had no remarkable medical history, having a height, weight, and head circumference judged to be within normal Peruvian limits, and being under thirty-six months of age.

The Bayley Infant Scales for Mental and Motor Development were translated into Spanish and used for testing mental development. The control children were found to be developing normally for their ages. This suggests, though the tests were made on a very small sample of children, that the Bayley Scales, as translated, are applicable to Peruvian children.

In contrast, all but two of the children who had been malnourished obtained developmental intelligence and motor quotients below the basal intelligence and motor quotients found in corresponding tables of the Bayley Mental and Motor Scales. This indicates severe mental and motor retardation despite apparent somatic recovery. In many test items these children were on the mental and motor scales at an age eight or more months behind their actual chronological age. There was shown, however, a degree of motor improvement relative to the duration of hospitalization...

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