small-pore (20 μ) filtration of aggregates of platelets, leucocytes, fibrin strands, and other debris.1

While dose–response data relating amount of microaggregate infusion to pulmonary damage have not been obtained, Connell and Swank have shown pulmonary microembolism with platelet-leukocyte microaggregates after blood transfusion using standard transfusion filters.3 These microemboli were associated with pulmonary capillary endothelial damage. Both the microemboli and the capillary damage were prevented by use of 20-μ-pore filtration. This survey suggests that blood stored less than five days contains much less potentially obstructive materials than older blood.

Our data indicate that at about the sixth day of storage some process producing a relatively sudden increase in screen filtration pressure occurs in blood. Further studies are under way to determine whether this process is fibrin formation. In addition, the survey suggests that studies of the effects of age and sex of the donor on pressure might help clarify the processes involved in the development of microaggregates and thus alter blood banking techniques to reduce the amounts of microaggregates and debris formed during storage.

REFERENCES

Insulin Adsorption by Glass Infusion Bottles, Polyvinylchloride Infusion Containers, and Intravenous Tubing

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The addition of insulin to intravenous fluids has been suggested for intraoperative management of the diabetic patient.1,2 This practice, however, has been precluded by the finding that insulin is adsorbed by glass and plastic.3,4 Adsorption of insulin to glass infusion bottles and plastic intravenous tubing at slow rates of infusion (two-hour period) has been documented.3 Adsortion ranged from 5 per cent for 20 units to 3.1 per cent for 40 units of insulin added to 500 ml isotonic saline solution, while plastic intravenous tubing adsorbed 30 per cent of 20 units and 26 per cent of 40 units of insulin added to the same infusion bottles. Rapid infusion of Ringer’s lactated solution or dextrose, 5 per cent, in Ringer’s lactate solution during the first hour of surgery is commonplace. The purpose of this study was to determine the amount of insulin adsorption during rapid infusion from glass bottles (1,000 ml), polyvinylchloride infusion containers, and intravenous tubing.

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METHODS

Containers and Infusion Fluids

Regular insulin (Iletin, Eli Lilly, beef-poor, U-40), 30 units, was injected into nine of each of the following: 1) 1,000-ml glass bottles of Ringer’s lactated solution; 2) 1,000-ml polyvinylchloride (Vialflex) containers of Ringer’s lactate solution; 3) 1,000-ml glass bottles of dextrose, 5 per cent, in Ringer’s lactate solution; 4) 1,000-ml polyvinylchloride containers of dextrose, 5 per cent, in Ringer’s lactate solution. The containers were immediately inverted and hung from stands. Samples (5 ml) of fluid were obtained immediately (0 time), and 5, 10, and 20 minutes after inversion using disposable 18-gauge 3 1/2-inch spinal needles. The concentration of insulin in each sample was determined in duplicate using a Phadebas (Pharmacia AB) radioimmunoassay insulin test kit.† Appropriate dilutions and overnight incubations were used to assure optimal recovery. Radioactivity was determined on a Packard gamma-counter using duplicate 5-minute counts. All samples were corrected for background noise. The volume of each infusion container was determined by subtracting the weight of the dried empty container from the pre-injection container weight. The exact amount of insulin injected was corrected for the volume of the spinal needle and deadspace of the 1-ml tuberculin syringe. Percentage of insulin recovered was determined for each container using the calculated amount of insulin injected and the measured recovered radioactivity concentrations in µU/ml.

Intravenous Tubing and Syringes

Insulin, 30 units, was injected into five glass containers of Ringer’s lactate solution. A Cutter adult intravenous-injection set was inserted into each container. Flow rate was adjusted to 14 ml/min and 5-ml samples were collected immediately (0 time) and at 5, 10, and 20 minutes. Each container was then opened and a 5-ml sample taken from the remaining mixture. All samples were analyzed in duplicate as described above.

The syringes used to collect insulin samples were studied to determine the amount of insulin lost during sample transfer from container to analysis tube. Six 5-ml syringes were filled from the same glass container of Ringer’s lactate solution to which 30 units of insulin had been added. A 0.2-ml sample was taken from each syringe at 0, 3, and 6 minutes and analyzed in duplicate. A separate sample was obtained from the glass container of Ringer’s lactate solution immediately following syringe sampling to serve as a 100 per cent control value.

† Radioactive insulin is bound by insulin antibody coupled by covalent linkages to a solid phase. Competitive inhibition occurs with unlabeled insulin and centrifugal separation allows measurement of the resultant insulin-antibody-solid phase complex.
Variable Insulin Concentrations

Regular insulin, 10 units, was added to ten glass containers of Ringer's lactate solution and samples were obtained at 0, 5, 10, and 20 minutes. Insulin, 20 units, was added to six glass containers of Ringer's lactate solution and samples were obtained at 0, 5, and 10 minutes. Injection sampling and analysis were the same as described above.

Prevention of Adsorption by Albumin and Plasma

The addition of albumin to prevent insulin adsorption is common laboratory practice. To four glass containers of Ringer's lactate solution, human serum albumin (Armour Pharmaceutical Co., 25 per cent solution), 1 ml, was added prior to the injection of insulin 30 units. Human serum albumin, 2 ml, was injected into four glass containers of Ringer's lactate solution. Following injection of insulin, all containers were sampled as described for the glass containers of Ringer's lactate solution containing 30 units.

Plasma protein fraction (Plasmanate, Cutter) has been used widely as a substitute for human serum albumin. The solution contains approximately 4.4 g albumin/100 ml. Following pilot studies, Plasmanate was injected into glass containers of Ringer's lactate solution, 60 ml each into 12 containers and 75 ml each into six containers. Insulin, 30 units, was added, and samples were obtained at 0, 5, and 10 minutes and analyzed. Insulin concentrations were corrected for the additional volumes of human serum albumin and Plasmanate.

RESULTS

Containers and Infusion Fluids

Maximal adsorption of insulin occurred in all containers during the 15-second period required for injection, inversion, and initial sampling. The loss of injected insulin dose at time 0 was 55 per cent. Comparison of time 0 with 5, 10, and 20 minutes using Student's t test revealed no statistical variation from time 0. Maximal adsorption had occurred at time 0 in all containers and did not change during the selected sampling period in any phase of the study.
Initial adsorption values at 5 minutes were chosen for comparisons of different types of infusion containers and different fluids. Adsorption losses (means ± SE) at 5 minutes were: Glass containers of Ringer's lactate solution, 52 ± 1.8 per cent; Polyvinylchloride containers of Ringer's lactate solution, 50 ± 1.4 per cent; Glass containers of dextrose, 5 per cent, and Ringer's lactate solution, 42 ± 2.0 per cent; Polyvinylchloride containers of dextrose, 5 per cent, and Ringer's lactate solution, 45 ± 2.4 per cent. (See fig. 1.)

Comparison of losses by adsorption showed significant differences: 1) glass containers of Ringer's lactate solution vs. dextrose, 5 per cent, and Ringer's lactate solution, $P < .001$; 2) polyvinylchloride containers of Ringer's lactate solution vs. dextrose, 5 per cent, and Ringer's lactate solution, $P < .001$; 3) dextrose, 5 per cent, and Ringer's lactate solution in glass vs. in polyvinylchloride containers, $P < .001$.

### Intravenous Tubing and Syringes

At a flow rate of 14 ml/min for 20 minutes, the adsorption of insulin by the intravenous tubing was 46 per cent at 0 time, 55 per cent at 5 minutes, 50 per cent at 10 minutes, and 48 per cent at 20 minutes (mean 50 per cent, ± 1.8). Combination of a glass container of Ringer's lactate solution with intravenous tubing (fig. 2) showed that when regular insulin, 30 units, was injected, 52 per cent (15.8 units) was immediately adsorbed to the container wall, and 55 per cent (7.9 units) of the remaining insulin in the container was adsorbed to the intravenous tubing wall, for a total adsorption of 79 per cent (23.7 units).

Syringes used for transfer adsorbed less than 7 per cent of the insulin sample. Maximal adsorption occurred at 0 time and did not change during the next 6 minutes.

### Variable Insulin Concentration

Maximal adsorption of insulin occurred during the first 15 seconds in glass containers of Ringer's lactate solution into which 10 or 20 units of insulin had been injected. No further adsorption occurred during the 20-minute period studied. Losses of insulin at 5 minutes from bottles containing 10, 20, and 30 units were 36 per cent (± 2.5 SE), 61 per cent (± 4.0 SE), and 52 per cent (± 1.8 SE), respectively. Comparison of 10-unit with 20- and 30-unit and 20-unit with 30-unit amounts of insulin proved significant, $P < .001$ and $P < .05$.

### Prevention of Adsorption by Albumin and Plasma

The addition of human serum albumin and Plasmanate was effective in decreasing the amount of insulin loss (fig. 3). Insulin adsorption was maximal during the first 15 seconds and did not change thereafter during the entire 20-minute period in bottles containing either human serum albumin or Plasmanate. The addition of human serum albumin, 1 ml, decreased the loss with 30 units of insulin from 52 to 28 per cent (± 1.5 SE), while human serum albumin, 2 ml, decreased it to only 36 per cent (± 2.1 SE). Plasmanate reduced the loss with 30 units of insulin from 52 to 32 per cent (± 1.4 SE) when 60 ml were tested and 34 per cent (± 3.0 SE) with 75 ml. Comparison of Plasmanate and human serum albumin values with the loss in the 30-unit regular Ringer’s lactate solution bottle showed
significant differences ($P < .001$ in all cases), but no significant difference was found for Plasmanate vs. human serum albumin.

**DISCUSSION**

It is apparent that there is considerable loss of insulin to intravenous fluid systems and intravenous tubing when clinical doses of insulin are added to infusion systems. Our results indicate that the insulin adsorption may be much greater than previously reported. Weinsenfeld et al.\(^5\) reported approximately 10 per cent adsorption to glass infusion bottles with 15 units of insulin in 500-ml infusion bottles. Using comparative doses of insulin (40 units in 1,000-ml infusion bottles), we found 45 to 52 per cent adsorption. Wiseman and Baltz\(^6\) found 21 per cent adsorption, while Frienkel and Goodner\(^2\) found 47 per cent adsorption in concentrations similar to those used in our studies. This means that of 30 units of insulin added to the infusion container, approximately 6.4 units reach the patient.

The marked variations in the results of studies of adsorption of insulin by glass are probably due to differences in methodology. In previous studies, known amounts of radioactive insulin were added to infusion systems or static systems and the amounts of insulin adsorbed determined by eluting the radioactive substances from the glass with a strong base (potassium hydroxide, 30 per cent). In our system, actual insulin concentrations were measured using radioimmunoassay techniques. Maximal adsorption of insulin is dependent on surface area.\(^5\) A one-liter infusion bottle has an internal surface area of approximately 695 sq cm, while a 500-ml bottle has a surface area of only 453 sq cm. The amounts of exposed surface area could account for some of the differences noted. The lack of linearity in adsorption among various doses of added insulin in our study and others\(^1,3\) is perplexing. No evidence to explain the decreased insulin adsorption seen in the presence of dextrose, 5 per cent, in both polyvinylchloride and glass containers is available.

Unlike investigators in prior studies,\(^1,5,6\) we were unable to show complete prevention of adsorption of insulin to glass by human serum albumin. Adsorption was decreased from 52 to 28 per cent. A pilot study failed to show any difference between concentrations of human serum albumin from 1 to 10 ml of 25 per cent solution. Plasmanate, 60 ml, was just as effective as 1 ml of human serum albumin in preventing insulin adsorption. Plasmanate is expensive but avoids the possibility of hepatitis.

In conclusion, we find that 55 per cent of insulin is adsorbed by glass and polyvinylchloride containers within 15 seconds of injection. The intravenous infusion set absorbs an additional 50 per cent of the insulin remaining in the intravenous fluid. Addition of 30 units of insulin to 1,000 ml of Ringer's lactate solution will result in the patient's receiving only 6.36 units of insulin because of losses by adsorption. This adsorption can be decreased by utilization of Plasmanate or human serum albumin. We recommend that insulin given intraoperatively be administered directly intravenously through a minimum length of tubing.

**REFERENCES**