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Metabolic and hormonal effects of glucagon infusion in erythroblastotic infants

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Milner, R. D. G., Chouksey, S. K., and Assan, R. (1973). *Archives of Disease in Childhood*, **48**, 885. **Metabolic and hormonal effects of glucagon infusion in erythroblastotic infants.** 10 or 50 μg glucagon was added to the bottle of donor blood, preserved with acid citrate and dextrose, used for exchange transfusion of erythroblastotic infants. The effects of the glucagon infusion on plasma glucose, insulin, growth hormone (GH), and glucagon were measured during the transfusion and for 60 minutes thereafter, and were compared with transfusions in which no addition was made to the donor blood. Both doses of glucagon produced similar effects: higher mean plasma glucose and insulin levels during the transfusion, but no significant change in plasma growth hormone levels. Transfusion with glucagon-enriched blood had no effect on the net balance of glucose or growth hormone, but caused a negative insulin balance twice as big as that occurring in the control group.

At the end of the control transfusions the mean plasma glucagon was 248 pg/ml and this did not change significantly in the next 60 minutes. When 10 or 50 μg glucagon was added to the donor blood the end-transfusion mean plasma glucagon concentrations were 1302 and 3975 pg/ml, respectively. Glucagon disappeared rapidly from plasma for 5 to 10 minutes and then more slowly at the rate of 0·5 to 1·0 % per minute for the next 50 minutes. The range of glucose disappearance rates in the 60 minutes after transfusion (0·40–2·13 %/min) was similar in all three groups, but the infants receiving glucagon-enriched blood had higher blood glucose levels for a given glucose disappearance rate. In each group there was a highly significant negative correlation between the 60-minute plasma glucose level and the glucose disappearance rate.

It is concluded that the addition of 10 or 50 μg glucagon to the bottle of donor blood used for exchange transfusion may have a protective effect against post-transfusion hypoglycaemia. Measurement of the blood glucose level 60 minutes after the transfusion has both diagnostic and prognostic value in detecting hypoglycaemia.

Glucagon stimulates hepatic glycogenolysis and insulin secretion. Recently, published reports have noted the beneficial effects of glucagon on cardiac function (Parmley, Glick, and Sonnenblick, 1968; Brogan, Kozonis, and Overy, 1969; Manchester *et al.*, 1970). Glucagon may therefore have a therapeutic place in the management of the erythroblastotic infants in whom heart failure is a common cause of death (Van Praagh, 1961). Since these babies hypersecrete insulin, it is apparent that glucagon might also make them hypoglycaemic.

The present study was undertaken to measure the metabolic and hormonal effects of glucagon in infants undergoing exchange transfusions to determine if any undesirable metabolic consequence might preclude the therapeutic use of the hormone as a cardiac stimulant in this procedure.

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Patients and methods

42 term newborn infants were studied. They were of normal weight for gestational age and suffered from mild to moderate erythroblastosis fetalis due to rhesus incompatibility. Results from 10 of the infants were reported earlier (Milner *et al.*, 1972). Certain clinical details are presented in Table I.

Exchange transfusions were performed using blood preserved with acid citrate and glucose (ACD) as described previously (Milner *et al.*, 1972). Immediately

TABLE I

Clinical details of infants studied

Type and no. of transfusions	No. and sex of patients		Mean ± SEM (range)			
	M	F	Birthweight (g)	Postnatal age (hr)	Time from last meal (min)	Rate of transfusion (ml/min)
ACD (25)	11	13	3022 ± 130 (2250–4020)	37 ± 7 (3–93)	221 ± 15 (60–480)	5·3 ± 0·2 (3·7–7·3)
ACD + 10μg glucagon (15)	6	6	2800 ± 144 (2250–3820)	36 ± 10 (4–126)	198 ± 16 (60–300)	5·5 ± 0·3 (4·2–8·1)
ACD + 50μg glucagon (10)	4	2	3150 ± 117 (2550–3570)	32 ± 7 (2–170)	109 ± 22 (72–210)	6·4 ± 0·3 (5·1–8·0)

ACD, blood preserved with acid citrate and glucose (Milner *et al.*, 1972).

before some transfusions, 10 or 50 μg glucagon (Eli Lilly) was added to the bottle of donor blood and mixed by inversion. The three types of transfusions are referred to as (i) ACD or control transfusions, (ii) ACD + 10 μg glucagon, and (iii) ACD + 50 μg glucagon. The infant was left in positive balance at the end of the transfusion so that the removal of blood samples in the following 60 minutes would produce the desired final haemodynamic balance. The end of the transfusion was defined as time 0. The umbilical venous catheter was left in place, flushed with heparinized saline (10 units/ml), and blood samples were withdrawn from it at 5, 10, 20, 30, 40, and 60 minutes. The total volume of these samples did not exceed 20 ml.

All blood was collected in chilled tubes containing 20 mg di-sodium ethylene diamine tetra-acetate and 4300 units kallikrein inhibitor (aprotinin) per 10 ml blood. Plasma was separated by centrifugation within 10 minutes and stored at 4 °C or −20 °C until analysed for glucose or insulin, glucagon, and growth hormone (GH) concentrations, respectively. Plasma glucagon concentrations were assayed at several dilutions and no effect of bilirubin on the assay was detected. Results were calculated and expressed as described previously (Milner *et al.*, 1972). Glucose disappearance rates (K_t) were calculated as described by Greville (1943).

Results

During transfusion. The donor blood in the three types of transfusions had similar plasma insulin and GH concentrations (Table II). The mean plasma glucose concentration in the transfusions with ACD + 10 μg glucagon was fortuitously higher than that in the other two groups. The addition of 10 or 50 μg glucagon to the donor blood raised the mean (±SE) plasma glucagon con-

TABLE II

Plasma concentrations (±SE) of glucose, insulin, GH, and glucagon during exchange transfusion with 3 different types of donor blood

	Donor	0 ml	100 ml	200 ml	300 ml	400 ml
Glucose (mg/100 ml)						
ACD	430 ± 12 (15)	70 ± 4 (15)	120 ± 4 (15)	143 ± 6 (15)	154 ± 5 (15)	159 ± 5 (15)
ACD + 10 μg glucagon	470 ± 7 (10)*	72 ± 3 (10)	153 ± 6 (10)†	172 ± 5 (10)†	185 ± 6 (10)†	195 ± 9 (10)†
ACD + 50 μg glucagon	418 ± 13 (15)	61 ± 2 (15)	131 ± 6 (15)	159 ± 6 (15)	175 ± 6 (15)*	185 ± 6 (15)†
Insulin (μU/ml)						
ACD	19 ± 2 (15)	26 ± 6 (15)	48 ± 8 (15)	53 ± 6 (15)	58 ± 10 (15)	66 ± 14 (15)
ACD + 10 μg glucagon	21 ± 2 (10)	29 ± 5 (10)	85 ± 17 (10)*	114 ± 19 (10)†	89 ± 14 (10)	81 ± 13 (10)
ACD + 50 μg glucagon	23 ± 3 (15)	25 ± 6 (15)	78 ± 12 (15)*	87 ± 15 (15)	92 ± 19 (15)	92 ± 20 (15)
GH (ng/ml)						
ACD	2·3 ± 0·4 (15)	34 ± 5 (15)	38 ± 7 (15)	53 ± 10 (15)	64 ± 11 (15)	72 ± 12 (15)
ACD + 10 μg glucagon	3·5 ± 0·3 (10)	71 ± 20 (10)*	59 ± 14 (10)	73 ± 17 (10)	91 ± 17 (10)	89 ± 14 (10)
ACD + 50 μg glucagon	2·5 ± 0·2 (15)	32 ± 4 (15)	33 ± 3 (15)	67 ± 9 (15)	92 ± 15 (15)	92 ± 16 (15)
Glucagon (pg/ml)						
ACD	80 ± 10 (13)	—	—	—	—	248 ± 33 (13)
ACD + 10 μg glucagon	7990 ± 1320 (10)‡	—	—	—	—	1302 ± 139 (9)‡
ACD + 50 μg glucagon	29680 ± 1600 (15)‡	—	—	—	—	3975 ± 315 (14)‡

Note: No. of observations in parentheses. Levels of significance for comparison with transfusions in which ACD blood was used: *P < 0·05, †P < 0·01, ‡P < 0·001.

centration from 80±10 pg/ml to 7990±1320 pg/ml and 29,680±1600 pg/ml, respectively. In the infants receiving ACD transfusions the plasma glucagon level after 400 ml was 248±33 pg/ml. In the transfusions performed with glucagon-enriched blood the levels were 5 and 16 times higher. The plasma glucose levels in the ACD+10 μg glucagon transfusions were significantly higher than those in the ACD transfusions from the 100 ml point onwards, whereas there was a significant difference in the ACD+50 μg glucagon transfusions in the 300 and 400 ml samples only. The difference may have been partly due to the higher donor blood glucose concentration in the ACD+10 μg glucagon transfusions. On the other hand, there was no significant linear correlation between the donor plasma glucose and the maximum plasma glucose in the infant during transfusion in any of the three groups. In both types of glucagon-enriched transfusion there was a higher plasma insulin level in the 100 ml sample than in the ACD transfusions. The difference persisted in the 200 ml sample of the ACD+10 μg glucagon transfusions, but thereafter similar plasma insulin levels were observed in all three types of transfusion. The only significant difference in plasma GH concentration was observed in the infants receiving transfusions with ACD+10 μg glucagon who had a higher mean plasma GH concentration at the start of the procedure.

The similarity of the effect of the two doses of glucagon on the plasma concentrations of glucose, insulin, and GH enabled results from the two groups to be pooled for the calculation of mean changes in plasma concentration (Table III). In this analysis results were included from 10 ACD transfusions performed earlier under comparable conditions. Glucagon caused a sustained rise in plasma glucose throughout the transfusion which was associated with higher plasma insulin levels during most of the procedure. Glucagon did not cause any difference in plasma GH levels.

Calculation of the total amount of glucose infused and withdrawn showed very similar results in all three types of transfusion (Table IV). Glucagon caused a net loss of insulin nearly twice that occurring in the control transfusions but had no significant effect on GH balance.

After transfusion. At the start of the post-transfusion period the mean plasma glucagon levels in the three groups of infants differed considerably (Table V). No change occurred in the mean plasma glucagon level of the control group in the next 60 minutes. The mean plasma glucagon level of both groups receiving glucagon-enriched blood fell rapidly in the first 5 to 10 minutes and more slowly thereafter (Fig. 1). The initial rate of fall was similar in the two groups, being 13·9% per minute in the infants who had received ACD+10 μg glucagon transfusions and 15·8% per minute in those who had ACD+50 μg glucagon transfusions. The subsequent disappearance rates were 0·5 and 1·0% per minute, respectively.

The mean plasma glucose of infants receiving ACD+50 μg glucagon transfusions remained significantly higher than that of the control group until the 60 minute sample. The mean plasma glucose level of the infants who had received ACD+10 μg glucagon transfusions were significantly raised at 5 and 10 minutes only. Hyperglycaemia and hyperglucagonaemia had no significant effect on plasma insulin and GH levels in the post-transfusion period. Mean plasma insulin levels in the ACD+10 μg glucagon group were similar to those in the control group, whereas those in the ACD+50 μg glucagon group tended to be

TABLE III

Changes in plasma concentrations (±SE) of glucose, insulin, and GH during exchange transfusion using ACD blood with or without glucagon

	100 ml	200 ml	300 ml	400 ml
Glucose (mg/100 ml)				
ACD	+48±2 (25)	+69±4 (25)	+82±5 (25)	+87±7 (25)
ACD + glucagon	+74±4 (25)*	+99±4 (25)*	+114±4 (25)*	+124±5 (25)*
Insulin (μU/ml)				
ACD	+25±7 (25)	+28±5 (25)	+35±8 (25)	+45±8 (23)
ACD + glucagon	+54±9 (25)	+71±10 (25)*	+64±11 (25)†	+65±13 (25)
GH (ng/ml)				
ACD	+9±5 (25)	+23±7 (25)	+39±10 (25)	+48±14 (23)
ACD + glucagon	+4±4 (25)†	+22±10 (25)	+44±11 (25)	+43±12 (25)

Note: Figures for ACD transfusions are from the results of present study plus results of Milner *et al.* (1972). Figures for ACD + glucagon transfusions calculated from transfusions in which 10 or 50 μg glucagon were added to donor blood. No. of observations in parentheses. Level of significance between the two types of transfusion: *P <0·001, †P <0·05.

TABLE IV

Total amount of metabolite or hormone infused or removed in 3 different types of transfusion

Hormone or metabolite	Amount infused or removed/kg body weight (Mean ± SEM)		
	In	Out	Balance
Glucose (mg)			
ACD (25)	252 ± 14	100 ± 4	+ 156 ± 12
ACD + 10 μg glucagon (10)	253 ± 9	103 ± 5	+ 151 ± 5
ACD + 50 μg glucagon (15)	267 ± 17	118 ± 8	+ 149 ± 14
Insulin (mU)			
ACD (25)	1·54 ± 0·19	3·90 ± 0·37	−2·34 ± 0·43
ACD + 10 μg glucagon (10)	1·13 ± 0·1	5·39 ± 0·65	−4·26 ± 0·67*
ACD + 50 μg glucagon (15)	1·57 ± 0·25	6·02 ± 1·08	−4·44 ± 0·98
GH (μg)			
ACD (25)	0·18 ± 0·27	4·25 ± 0·44	−4·07 ± 0·44
ACD + 10 μg glucagon (10)	0·36 ± 0·03	5·00 ± 1·07	−4·65 ± 1·10
ACD + 50 μg glucagon (15)	0·16 ± 0·16	5·07 ± 0·63	−4·90 ± 0·63

Note: No. of observations in parentheses. Level of significance for comparison with transfusions in which ACD blood was used: *P < 0·05.

higher. Plasma GH levels were similar in all three groups.

Glucose disappearance rates (K_t) were calculated for each infant. The mean (±SE) K_t for the ACD transfusion group was 1·30 ± 0·15 (range 0·40–1·58). This did not differ significantly from the mean value of the ACD + 10 μg glucagon group, 1·47 ± 0·17 (range 0·50–2·13), nor from that of the ACD + 50 μg glucagon group, 1·19 ± 0·21 (range 0·59–1·93). In Fig. 2 plasma glucose levels 5 minutes after the transfusion are plotted as a scatterdiagram against K_t. There is no significant correlation between the two variables, but the higher plasma glucose concentrations in the infants who had received glucagon-enriched blood is apparent. In contrast, the plasma glucose 60 minutes after the transfusion correlated negatively with K_t in both control and experimental groups (Fig. 3). The negative correlation between plasma glucose and K_t is highly significant in both ACD transfusions ($r = 0·912$, $P < 0·001$) and in glucagon-enriched transfusion ($r = 0·878$, $P < 0·001$). No significant correlation between K_t and 5- or 60-minute plasma insulin or GH levels was noted.

Discussion

The present study has shown that glucagon stimulates insulin secretion when added to the donor blood used in exchange transfusions. The increased secretion was manifest both as a rise in plasma concentration during the transfusion and as an increased net loss of insulin resulting from the procedure, but had no untoward effect as judged clinically or by the measurement of plasma glucose

TABLE

Plasma concentrations (±SE) of glucose, insulin, GH, and glucagon

	0 min	5 min	10 min
Glucose (mg/100 ml)			
ACD	159 ± 5 (15)	127 ± 5 (10)	116 ± 6 (10)
ACD + 10 μg glucagon	195 ± 9 (10)†	161 ± 13 (10)*	146 ± 10 (10)*
ACD + 50 μg glucagon	185 ± 6 (15)†	173 ± 9 (5)†	157 ± 11 (6)*
Insulin (μU/ml)			
ACD	66 ± 14 (15)	107 ± 29 (10)	90 ± 17 (10)
ACD + 10 μg glucagon	81 ± 12 (10)	117 ± 21 (10)	95 ± 14 (10)
ACD + 50 μg glucagon	92 ± 20 (15)	99 ± 26 (6)	116 ± 27 (6)
GH (ng/ml)			
ACD	72 ± 12 (15)	79 ± 12 (10)	80 ± 14 (10)
ACD + 10 μg glucagon	89 ± 14 (10)	84 ± 14 (10)	81 ± 16 (10)
ACD + 50 μg glucagon	92 ± 16 (15)	86 ± 25 (5)	85 ± 20 (6)
Glucagon (pg/ml)			
ACD	248 ± 33 (13)	245 ± 32 (10)	249 ± 36 (10)
ACD + 10 μg glucagon	1302 ± 146 (9)‡	689 ± 128 (10)†	506 ± 65 (10)†
ACD + 50 μg glucagon	3975 ± 327 (14)‡	1776 ± 277 (6)‡	804 ± 33 (7)‡

Note: No. of observations in parentheses. Level of significance for comparison with transfusions in which ACD blood was used: *P < 0·05, †P

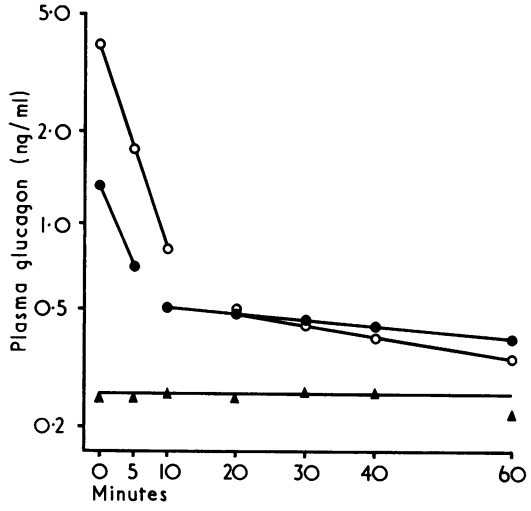

FIG. 1.—*Mean plasma glucagon concentration after exchange transfusion in infants who received ACD transfusions* ▲, *ACD+10 μg glucagon transfusions* ●, *or ACD+50 μg glucagon transfusions* ○.

levels. Higher rises in plasma glucose occurred throughout the glucagon-enriched transfusions and persisted for 40 minutes afterwards in infants who had received ACD+50 μg glucagon transfusions. These changes were probably due to stimulation by glucagon of hepatic glycogenolysis coincident with the glucose infusion, since the net positive glucose balance in each of the three groups was similar.

The overall effect on glucose homoeostasis of adding glucagon to the donor blood may be best appreciated by considering the rate of glucose disappearance in the hour after the transfusion. The range of K_t was similar in control and experimental groups, but for a given K_t the 60-minute plasma glucose was higher in infants who had received glucagon-enriched blood (Fig. 3). The reason for this is not clear, since the infants receiving ACD+50 μg glucagon transfusions also had higher mean plasma insulin levels in the post-transfusion period. Hyperglucagonaemia persisted throughout the 60-minute period in both groups receiving glucagon-enriched blood and might have counteracted the action of insulin. Plasma GH levels remained raised in all three groups, suggesting that increased secretion of GH was not responsible for the similarity of the K_t values. Though the results show a protective effect of glucagon against hypoglycaemia in the first hour after transfusion, they do not indicate if the protection would persist during the second and third hours when hypoglycaemia most commonly occurs (Schiff *et al.*, 1971).

The close negative correlation between K_t and the 60-minute post-transfusion plasma glucose level suggests that the measurement of the blood glucose concentration one hour after the transfusion has both diagnostic and prognostic value. The lower the blood glucose at this time the faster the rate of glucose disappearance and the more likely is the baby to become hypoglycaemic.

Glucagon stimulates GH secretion in normal newborn infants (Milner and Wright, 1967), but the infants who received glucagon-enriched donor blood did not secrete more GH than the control group. Increased GH secretion in ACD transfusions has been shown to be due in part to the glucose infusion and in part to other factors (Milner *et al.*, 1972).

V

after exchange transfusion with 3 different types of donor blood

20 min	30 min	40 min	60 min
109±7 (10)	97±6 (10)	86±6 (9)	78±6 (10)
125±10 (10)	107±11 (10)	93±10 (10)	75±9 (10)
145±9 (6)*	132±9 (6)*	117±8 (6)*	94±10 (6)
88±24 (10)	75±20 (10)	50±15 (9)	53±16 (10)
77±15 (10)	61±14 (10)	54±15 (10)	59±19 (10)
123±26 (6)	137±31 (6)	104±24 (6)	81±24 (6)
73±12 (10)	67±13 (10)	62±12 (9)	64±13 (10)
74±13 (10)	72±12 (10)	74±13 (10)	76±12 (10)
78±18 (6)	80±16 (6)	81±16 (6)	96±15 (6)
241±27 (10)	258±27 (10)	252±28 (9)	215±19 (10)
497±61 (10)‡	445±63 (10)†	444±50 (10)†	393±52 (10)†
488±57 (6)†	428±49 (7)†	391±40 (7)*	327±52 (6)

·01, ‡P <0·001.

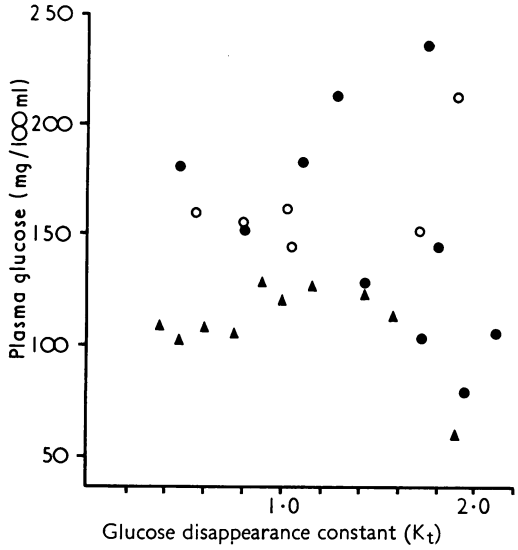

FIG. 2.—*Scatterdiagram of plasma glucose 5 minutes after exchange transfusion plotted against K_t. Symbols as in Fig. 1.*

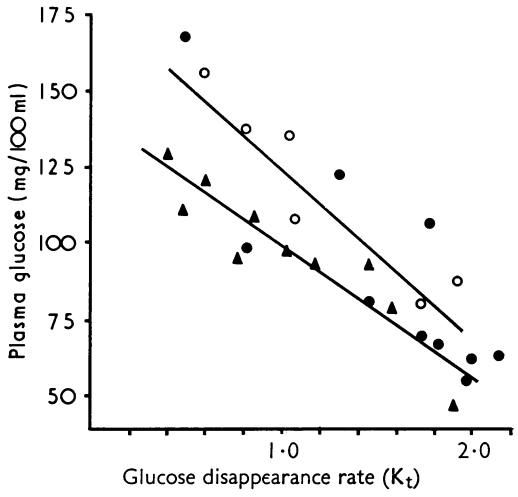

FIG. 3.—*Scatterdiagram of plasma glucose 60 minutes after exchange transfusion plotted against K_t. Symbols as in Fig. 1. The lower line shows the linear relation between plasma glucose and K_t in the ACD transfusions and the upper line the same relation in glucagon-enriched transfusions.*

The failure of glucagon to increase GH secretion further suggests that GH release during an ACD transfusion may be occurring at a maximum rate. The persistence of high plasma GH levels for 60 minutes after the transfusion indicates that the hypersecretion of GH continues for some time.

The amount of glucagon added to the donor blood initially, 50 μg, was chosen empirically since it was known that glucagon is rapidly destroyed in the presence of blood (Heding, 1971; Assan, 1972). This proved to be the case, since the mean plasma glucagon level in the donor bottle at the start of the transfusion was 29·68 ng/ml, whereas if the hormone had been undamaged the plasma concentration would have been in the order of 250 ng/ml. Further destruction of glucagon undoubtedly occurred *in vitro* during the transfusion since the mean plasma concentration in the infants at the end of the procedure was only 13% of that in the donor bottle. Similar results were obtained when 10 μg glucagon was added to the donor blood. No explanation is apparent for the significantly lower mean plasma glucagon level in the donor ACD blood than that reported previously (Milner *et al.*, 1972).

The disappearance of radioactive glucagon (Berson, Yalow, and Volk, 1957) or exogenous pancreatic glucagon (Assan, 1972) from the circulation in adults is rapid, and occurs at a similar rate to that observed in the first 10 minutes of the post-transfusion period. The subsequent apparently slow disappearance of the hormone is open to different interpretations. It is possible that the initial rate of glucagon loss was unphysiological and due in part to hepatic and renal catabolism and excretion (Assan, 1972), whereas the slower subsequent rate of disappearance was physiological and indicated that the newborn infant has a reduced ability, as with many other substances, to catabolize glucagon. An alternative explanation is that the initial rate of loss was physiological and the subsequent apparent slow disappearance was due to the persistence of immunoreactive glucagon fragments in the serum (Heding, 1971). It is unlikely that the high plasma concentrations of glucagon from 20 to 60 minutes post-transfusion were due to endogenous glucagon secretion since the infants were both hyperglycaemic and hyperinsulinaemic, conditions known to suppress glucagon release (Müller *et al.*, 1970).

When the effects of 50 μg glucagon on plasma glucose and insulin had been shown, further transfusions were performed using 10 μg to see if a different response occurred. The only significant difference between the two doses was that the infants receiving 50 μg glucagon remained hyperglycaemic for longer after the transfusion ended. For this reason it may be suggested that the higher dose had a more beneficial clinical effect.

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