Effect of Glucose on Plasma Glucagon, Growth Hormone, and Insulin in Exchange Transfusion

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Milner, R. D. G., Fekete, M., Assan, R., and Hodge, J. S. (1972). *Archives of Disease in Childhood*, 47, 179. **Effect of glucose on plasma glucagon, growth hormone, and insulin in exchange transfusion.** Exchange transfusions were performed on term normal-for-dates, and term small-for-dates infants using blood preserved with acid citrate and glucose or acid citrate alone. The plasma concentrations of glucose, free fatty acids, glycerol, insulin, glucagon, and growth hormone were measured in the donor blood and in blood from the infant at different times during the transfusion. The total amounts of the metabolites and hormones infused and removed from the infant were calculated. The infusion of glucose in blood preserved with acid citrate and glucose caused a rise in plasma glucose, insulin, and growth hormone levels and a fall in plasma free fatty acid levels. The plasma glycerol levels did not change significantly during transfusions with blood preserved with acid citrate and glucose, or acid citrate alone. In both types of exchange transfusion there was a similar gradual fall in plasma glucagon concentration and net loss of free fatty acid, glycerol, and glucagon. A net loss of growth hormone occurred which was greater in transfusions performed with blood preserved with acid citrate and glucose than in transfusions performed with blood preserved with acid citrate. In transfusions performed with blood preserved with acid citrate and glucose there was a net accumulation of glucose by the infant and a net loss of insulin.

Study of the metabolic and hormonal changes in exchange transfusions has clinical importance and biological interest. This essential therapeutic procedure is still associated with an appreciable mortality, the cause of which is not always clear (Weldon and Odell, 1968). It is important to analyse further the profound metabolic and hormonal changes known to occur during exchange transfusion (see Anderson *et al.*, 1963; Calladine *et al.*, 1965; Milner and Wright, 1966; Schiff *et al.*, 1971) in order to clarify and reduce the risk experienced by the infant during this procedure. Though the infants studied have erythroblastosis fetalis and are intrinsically a heterogeneous group at the time of transfusion, it is possible, by paying careful attention to such variables as gestational age, birthweight, postnatal age, and technique of transfusion, to select comparable groups. If the controlled difference between 2 such groups is chosen carefully, observations can be made which

are pertinent to the optimal management of infants with erythroblastosis and which may be of general biological relevance to the normal newborn infant.

The effect of glucose in blood preserved with acid citrate and glucose has been studied in 2 groups of term infants, one of normal birthweight and the other small-for-dates.

Patients and Methods

Two groups of infants of 37–40 weeks' gestational age were studied: 13 had a normal birthweight and 10 were small for their gestational age judged by the criteria of Butler and Alberman (1969). Clinical details of the 2 groups of infants are presented in Table I. Exchange transfusions were performed because of erythroblastosis due to rhesus incompatibility. The pregnancies and deliveries were otherwise uneventful. No infant was hydropic. The erythroblastosis was mild or moderate and was similar in the different groups studied. With one exception, no infant was studied twice within one group.

Two types of exchange transfusion were performed. Blood preserved with acid citrate and glucose (ACD),

TABLE I
Clinical Details of Infants and Exchange Transfusion Technique

Clinical Group and No. of Exchanges	No. of Patients and Sex		Mean $\pm$ SEM (and range)				
	M	F	Birthweight (g)	Postnatal Age (hr)	Time from Last Meal (min)	Rate of Exchange (ml/min)	Volume Exchanged/ Blood Vol. of Infant
Normal-for-dates, acid citrate dextrose blood (10)	7	3	3144 $\pm$ 144 (2550-4020)	35 $\pm$ 8 (9-93)	247 $\pm$ 28 (170-480)	4.9 $\pm$ 0.3 (3.7-7.3)	1.5 $\pm$ 0.06 (1.2-1.8)
Normal-for-dates, acid citrate blood (7)	3	4	3324 $\pm$ 199 (2680-4020)	55 $\pm$ 9 (4-76)	227 $\pm$ 35 (180-300)	5.5 $\pm$ 0.5 (3.6-8.3)	1.4 $\pm$ 0.09 (1.0-1.7)
Small-for-dates, acid citrate dextrose blood (6)	3	2	2506 $\pm$ 66 (2340-2700)	42 $\pm$ 18 (7-120)	205 $\pm$ 36 (60-420)	5.4 $\pm$ 0.3 (4.6-6.7)	1.8 $\pm$ 0.08 (1.5-2.1)
Small-for-dates, acid citrate blood (7)	2	5	2417 $\pm$ 35 (2080-2700)	63 $\pm$ 12 (16-114)	222 $\pm$ 18 (150-300)	6.2 $\pm$ 0.5 (4.2-8.0)	1.8 $\pm$ 0.07 (1.6-2.0)

supplied by the Blood Transfusion Service, less than 3 days old, semipacked to a haematocrit of 50-60% was used in normal and small-for-dates infants. These 2 groups are referred to as NFD-ACD and SFD-ACD, respectively. Special collection bottles (kindly prepared by Dr. F. Stratton, Manchester Regional Hospital Board, National Blood Transfusion Service) containing acid citrate only (AC) were used to collect blood from donors immediately before a transfusion was performed. After crossmatching, this blood was semipacked similarly. The 2 groups of infants receiving AC blood are referred to as NFD-AC and SFD-AC. ACD bottles contained 2 g disodium citrate and 3 g dextrose dissolved in 120 ml water; AC bottles contained 2.21 g disodium citrate dissolved in 70 ml water.

The exchange transfusions were performed by umbilical vein catheterization through which blood was withdrawn and injected in 20 ml aliquots. The catheter was inserted the minimum distance for easy withdrawal of blood, usually 4-6 cm. The infant was transfused on an 'Infant Warmer' (Air-Shields) set so that the ambient temperature was between 30 and 35.5 °C. The donor blood passed through a 700 cm plastic coil (Hem-o-gard Blood Warming Coil) immersed in water at 36.5-38.5 °C *en route* to the infant. The right midaxillary skin temperature was recorded continuously with a temperature monitor (Air-Shields) and was between 34.0 and 37.4 °C at the start of the transfusion. No significant change was noted in the mean ambient temperature and the mean temperature of the water in the different groups of infants studied. The mean skin temperatures of the different groups of infants were not significantly different at the start of the transfusion and did not alter significantly during the transfusion. Transfusions were performed at a wide range of postnatal ages, but there was little difference in the mean postnatal age at transfusion between groups. The mean rate of transfusion and the mean time of the transfusion after the last feed were similar in the different groups. The blood volume of the infant was calculated as 85 ml/kg body weight and the volume of blood exchanged was expressed as a fraction of the blood

volume of the infant (Table I). The volume exchanged was similar in the 2 NFD and the 2 SFD groups.

Blood specimens were collected in heparinized tubes from the donor bottle at the start of the exchange, from the infant before the injection of donor blood, and after the injection of 100, 200, 300, and 400 ml. Waste blood was collected in a heparinized measuring cylinder. At the end of the transfusion the volume of waste blood was measured and a specimen was collected for analysis. The haematocrit of all blood specimens was measured immediately and the plasma was then separated by centrifugation within 10 minutes. Aliquots of plasma were stored at -20 °C for hormone analysis. Plasma metabolite concentrations were measured within 24 hours on specimens stored at +4 °C. Plasma specimens for hormone analysis were thawed once only.

Plasma glucose was measured using glucose oxidase (Trinder, 1969), plasma FFA by the method of Duncombe (1964), and plasma glycerol by the method of Eggstein and Kreutz (1966) using reagents from Boehringer, Mannheim, G.m.b.H.

Plasma insulin was measured by immunoassay (Hales and Randle, 1963) using a human insulin standard (MRC preparation 66/304). Plasma growth hormone was measured by immunoassay using 'growth hormone binding reagent' (Wellcome Reagents Ltd., Beckenham), filtration for the separation of 'bound' and 'free' hormone, and a human growth hormone standard (MRC preparation 66/217). Plasma glucagon was measured by the method of Assan, Tchobroutsky, and Derot (1971), using a pork glucagon standard (kindly given by Dr. Bouchet of Novo, Paris) and an antibody to glucagon-like immunoreactive material which cross-reacted weakly with gut glucagon.

Calculation and expression of results. In each of the 4 groups of exchange transfusions the mean and standard error of mean plasma concentration of different metabolites and hormones were calculated for the donor blood and blood from the infant at the start of the transfusion and after the injection of 100, 200, 300, and 400 ml donor blood. A statistical comparison between

TABLE II

Plasma Metabolite and Hormone Concentrations in Normal Term Infants During Exchange Transfusions Using Blood Preserved with Acid Citrate and Glucose, or Acid Citrate Alone

Metabolite or Hormone	Mean $\pm$ SEM Plasma Concentration During the Transfusion					
	Donor	0 ml	100 ml	200 ml	300 ml	400 ml
Acid citrate blood						
Glucose (mg/100 ml)	89 $\pm$ 3 (7)	83 $\pm$ 5 (7)	83 $\pm$ 4 (7)	85 $\pm$ 4 (7)	82 $\pm$ 5 (7)	79 $\pm$ 5 (4)
FFA (μ mol/l.)	268 $\pm$ 38 (7)	883 $\pm$ 160 (7)	805 $\pm$ 153 (7)	743 $\pm$ 135 (7)	723 $\pm$ 127 (7)	631 $\pm$ 248 (4)
Glycerol (μ mol/l.)	55 $\pm$ 11 (7)	88 $\pm$ 12 (7)	88 $\pm$ 17 (7)	69 $\pm$ 11 (7)	83 $\pm$ 21 (7)	79 $\pm$ 32 (4)
Insulin (μ U/ml)	24 $\pm$ 4 (7)	26 $\pm$ 4 (7)	29 $\pm$ 3 (7)	26 $\pm$ 4 (7)	25 $\pm$ 2 (7)	19 $\pm$ 1 (4)
Glucagon (pg/ml)	357 $\pm$ 30 (5)	469 $\pm$ 84 (5)	453 $\pm$ 63 (5)	389 $\pm$ 63 (5)	425 $\pm$ 57 (5)	358 $\pm$ 31 (3)
GH (ng/ml)	3.0 $\pm$ 0.8 (7)	33 $\pm$ 12 (7)	28 $\pm$ 9 (7)	25 $\pm$ 5 (7)	24 $\pm$ 6 (7)	27 $\pm$ 8 (4)
Acid citrate dextrose blood						
Glucose (mg/100 ml)	386 $\pm$ 35 (10)*	75 $\pm$ 4 (10)	120 $\pm$ 3 (10)*	139 $\pm$ 4 (10)*	153 $\pm$ 8 (10)*	157 $\pm$ 14 (8)‡
FFA (μ mol/l.)	302 $\pm$ 47 (10)	1299 $\pm$ 155 (10)	983 $\pm$ 80 (10)	817 $\pm$ 72 (10)	596 $\pm$ 70 (10)	503 $\pm$ 70 (8)
Glycerol (μ mol/l.)	63 $\pm$ 11 (10)	114 $\pm$ 23 (10)	94 $\pm$ 17 (10)	91 $\pm$ 15 (10)	97 $\pm$ 19 (10)	88 $\pm$ 21 (8)
Insulin (μ U/ml)	37 $\pm$ 6 (10)	34 $\pm$ 6 (10)	64 $\pm$ 17 (10)	63 $\pm$ 10 (10)†	74 $\pm$ 17 (10)†	81 $\pm$ 15 (8)†
Glucagon (pg/ml)	282 $\pm$ 29 (10)	513 $\pm$ 80 (10)	433 $\pm$ 66 (10)	423 $\pm$ 62 (10)	410 $\pm$ 54 (10)	377 $\pm$ 36 (8)
GH (ng/ml)	3.0 $\pm$ 0.7 (10)	29 $\pm$ 4 (10)	45 $\pm$ 8 (10)	58 $\pm$ 9 (10)†	84 $\pm$ 16 (10)†	94 $\pm$ 31 (8)

Level of statistical significance for comparison of ACD blood versus AC blood: * $P > 0.001$, † $P > 0.005$, ‡ $P > 0.05$. Number of observations are shown in parentheses.

mean values was made by Student's 't' test. The significance of the mean difference between paired values was tested similarly.

The net balance of the different hormones and metabolites transfused in and out of the infant was calculated. The total amount of a substance in the plasma of the donor blood given to the infant was estimated from the expression: $T = VC(100 - H)/100$, where T was the total amount of the substance infused, V was the total blood volume infused, C was the plasma concentration of the substance, and H was the haematocrit. The total amount of a substance removed from the infant was calculated similarly, paying careful attention to the volume, haematocrit, and plasma concentration of the substance in the waste blood and each of the blood samples taken from the infant for analysis. If more of a hormone or metabolite was infused than was removed, the infant was described as being in positive balance and *vice versa*.

Results

Two types of exchange transfusion (ACD and AC) were performed on 2 clinical groups of infants (NFD and SFD). Since similar results were obtained with ACD and AC transfusions in each clinical group, the results for the NFD groups only are described in detail (Tables II and in part of Table IV, Fig. 1 and 2).

Donor blood. The donor blood for the NFD infants receiving ACD or AC transfusions was similar in all metabolic and hormonal respects with the exception of the plasma glucose concentration. The mean ($\pm$ SE) haematocrit of the ACD donor blood was $58 \pm 3\%$ and of the AC blood $54 \pm 2\%$.

Glucose. During the ACD transfusions the plasma glucose of the infants rose progressively while in the AC transfusions no significant change

FIG. 1.—Changes in the mean plasma concentration of glucose, FFA, insulin, and GH during exchange transfusions using blood preserved with acid citrate and dextrose (ACD) or acid citrate alone (AC) in term rhesus-affected infants of normal body weight.

TABLE III
Plasma Metabolite and Hormone Concentrations in Small-for-dates Term Infants During Exchange Transfusions Using Blood Preserved with Acid Citrate and Glucose or Acid Citrate Alone

Metabolite or Hormone	Mean $\pm$ SEM Plasma Concentration During Transfusion					
	Donor	0 ml	100 ml	200 ml	300 ml	400 ml
<i>Acid citrate blood</i>						
Glucose (mg/100 ml)	92 $\pm$ 6 (7)	78 $\pm$ 5 (7)	82 $\pm$ 5 (7)	84 $\pm$ 5 (7)	82 $\pm$ 5 (7)	89 $\pm$ 6 (4)
FFA (μ mol/l.)	354 $\pm$ 53 (7)	897 $\pm$ 113 (7)	784 $\pm$ 73 (7)	756 $\pm$ 82 (7)	725 $\pm$ 82 (7)	594 $\pm$ 119 (4)
Glycerol (μ mol/l.)	39 $\pm$ 11 (7)	60 $\pm$ 12 (7)	54 $\pm$ 16 (7)	64 $\pm$ 23 (7)	58 $\pm$ 27 (7)	39 $\pm$ 11 (4)
Insulin (μ U/ml)	31 $\pm$ 6 (7)	23 $\pm$ 4 (7)	25 $\pm$ 6 (7)	22 $\pm$ 2 (7)	22 $\pm$ 4 (7)	24 $\pm$ 4 (4)
Glucagon (pg/ml)	282 $\pm$ 49 (5)	724 $\pm$ 119 (5)	578 $\pm$ 86 (5)	457 $\pm$ 47 (5)	484 $\pm$ 59 (5)	455 $\pm$ 57 (3)
GH (ng/ml)	3.0 $\pm$ 0.7 (7)	41 $\pm$ 5 (7)	45 $\pm$ 4 (7)	42 $\pm$ 5 (7)	39 $\pm$ 5 (7)	47 $\pm$ 5 (4)
<i>Acid citrate dextrose blood</i>						
Glucose (mg/100 ml)	522 $\pm$ 48 (6)*	76 $\pm$ 11 (6)	120 $\pm$ 18 (6)	160 $\pm$ 17 (6)†	180 $\pm$ 17 (6)*	172 $\pm$ 18 (4)‡
FFA (μ mol/l.)	262 $\pm$ 44 (5)	996 $\pm$ 134 (5)	917 $\pm$ 131 (5)	724 $\pm$ 90 (5)	521 $\pm$ 54 (5)	411 $\pm$ 58 (4)
Glycerol (μ mol/l.)	53 $\pm$ 20 (5)	123 $\pm$ 37 (5)	83 $\pm$ 13 (5)	73 $\pm$ 9 (5)	80 $\pm$ 20 (5)	84 $\pm$ 14 (4)
Insulin (μ U/ml)	32 $\pm$ 2 (6)	27 $\pm$ 5 (6)	58 $\pm$ 5 (6)†	82 $\pm$ 18 (6)*	70 $\pm$ 18 (6)*	103 $\pm$ 34 (4)
Glucagon (pg/ml)	—	—	—	—	—	—
GH (ng/ml)	2.0 $\pm$ 0.6 (5)	41 $\pm$ 8 (5)	45 $\pm$ 6 (5)	63 $\pm$ 9 (5)	84 $\pm$ 15 (5)	84 $\pm$ 18 (4)

Level of statistical significance for comparison of ACD blood versus AC blood: *P < 0.001, †P < 0.005, ‡P < 0.05. Number of observations shown in parentheses.

FIG. 2.—Mean ($\pm$ SE) absolute amounts of glucose, FFA, glycerol, insulin, GH, and glucagon injected into and removed from term rhesus-affected infants treated by exchange transfusion using blood preserved with acid citrate and dextrose (ACD) or acid citrate alone (AC).

occurred. In the ACD transfusions there was a significant positive balance of glucose, but not in the AC transfusions.

Insulin. The rise in plasma glucose and the retention of glucose in the ACD transfusions was associated with a rise ($P < 0.01$) in the mean plasma insulin level from the 200 ml sample onwards. No significant change occurred during the AC transfusions. In ACD transfusions approximately twice as much insulin was removed from the infant as was infused, while no significant change in insulin balance occurred in AC transfusions.

Growth hormone. The mean plasma GH concentration in the donor blood in both ACD and AC transfusions was much lower than that in the infant at the start of the transfusion. Despite this difference, there was a progressive rise in the

Total Amount of Metabolite or Hormone Infused or Removed from Infants During Exchange Transfusion

Hormone or Metabolite	Normal-for-dates Infants			
	ACD Blood		AC Blood	
	In	Out	In	Out
Glucose (mg)	671 $\pm$ 65*	308 $\pm$ 19 (10)	167 $\pm$ 7	187 $\pm$ 11 (6)
FFA (μ mol)	52.6 $\pm$ 7.7*	193.9 $\pm$ 23.7 (10)	51.6 $\pm$ 9.0†	164.4 $\pm$ 31.1 (7)
Glycerol (μ mol)	12.0 $\pm$ 3.0†	21.9 $\pm$ 3.6 (10)	10.4 $\pm$ 2.3	19.2 $\pm$ 3.8 (6)
Insulin (mU)	6.71 $\pm$ 1.46†	13.03 $\pm$ 1.92 (10)	5.03 $\pm$ 1.08	6.14 $\pm$ 0.79 (6)
Glucagon (ng)	51.9 $\pm$ 7.0†	90.3 $\pm$ 11.8 (10)	68.7 $\pm$ 6.5	93.3 $\pm$ 13.0 (5)
GH (μ g)	0.64 $\pm$ 0.16*	14.3 $\pm$ 2.3 (10)	0.59 $\pm$ 0.17†	5.62 $\pm$ 1.43 (7)

Figures in parentheses indicate the number of pairs of observations.

The amount infused differs statistically from the amount removed: *P < 0.001, †P < 0.005, ‡P < 0.05.

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mean plasma GH concentration of the infant in the ACD transfusions which was significant from 100 ml onwards. In the AC transfusions the plasma GH level of the infant remained steady. In both ACD and AC transfusions there was a large negative balance of GH. The mean negative balance of GH in ACD transfusions was $13.6 \pm 2.3 \mu\text{g}$ which was significantly greater than that in AC transfusions: $5.0 \pm 1.4 \mu\text{g}$ ($P < 0.02$).

Free fatty acids. In ACD and AC transfusions there was a similar mean negative balance in FFA. The mean difference between the plasma FFA of the infant and that of the donor blood at the start of ACD transfusions was $986 \pm 127 \mu\text{mol/l.}$, which was not significantly greater than that in AC transfusions, $643 \pm 166 \mu\text{mol/l.}$ During the ACD and AC transfusions the mean fall in plasma FFA between the first and last samples from the infant was $745 \pm 138 \mu\text{mol/l.}$ and $170 \pm 63 \mu\text{mol/l.}$, respectively. The fall in plasma FFA concentration in ACD transfusions was significantly greater than that in AC transfusions ($P < 0.01$).

Glycerol. The mean plasma glycerol level in the infants receiving either type of transfusion was slightly but not significantly higher than that in the donor blood. During the transfusions no significant change in plasma glycerol occurred. In both groups there was a similar negative glycerol balance.

Glucagon. In both types of transfusion there was a similar negative glucagon balance, which was significant, however, only in the ACD transfusions. In both groups the mean donor plasma glucagon level was less than that in the infant at the start of the transfusion, but, again, the difference was significant only in the ACD group ($P < 0.02$). During the transfusion there was a gradual fall in

the mean plasma glucagon in both groups and in the last sample from infants having ACD transfusions the plasma glucagon was significantly lower than at the start of the transfusion ($P < 0.02$).

Small-for-dates group. The results from the AC and ACD transfusions in the SFD group are presented in Table III. In all respects the results from the SFD and NFD groups were qualitatively and quantitatively similar. In some cases changes in plasma concentrations or balances of metabolites or hormones were not significant in the SFD group when significant differences were found in the NFD group. This was due mainly to the group sizes.

Discussion

This study of the effect of glucose on the metabolic and hormonal changes in exchange transfusion was prompted by the desire to investigate the effect of glucose on glucagon secretion in the human newborn and to reinvestigate the effect of glucose infusion on GH secretion. Milner and Wright (1966) observed that plasma GH levels did not fall during exchange transfusions despite the fact that the plasma concentration of GH in the infant was more than 10 times that in the donor blood. They inferred that increased GH secretion occurred to make good the GH lost in the transfusion and that glucose was the stimulus to GH secretion. Others (Cornblath *et al.*, 1965; Westphal, 1968; Wolf, Stubbe, and Šabata, 1970) also found that hyperglycaemia in the newborn was associated with a rise in plasma GH levels, and deduced that the relation was causal. More recently emphasis has been placed on the fact that stress is a potent stimulus of GH release in older children (Helge, Weber, and Quabbe, 1969) and the possibility exists that the rise in plasma GH in the newborn after intravenous glucose is artefactual. Against

Transfusion with Blood Containing Acid Citrate and Glucose, or Acid Citrate Alone

Small-for-dates Infants			
ACD Blood		AC Blood	
In	Out	In	Out
$841 \pm 115\ddagger$	$311 \pm 28 (6)$	162 ± 20	$165 \pm 18 (7)$
$45.8 \pm 8.7\ddagger$	$169.8 \pm 27.5 (5)$	$61.8 \pm 10.7^*$	$153.2 \pm 12.5 (7)$
$9.0 \pm 3.5\ddagger$	$19.8 \pm 4.1 (6)$	6.5 ± 1.7	$12.2 \pm 3.2 (7)$
$5.23 \pm 0.85\ddagger$	$12.30 \pm 2.23 (6)$	5.08 ± 0.93	$4.20 \pm 0.55 (7)$
—	—	53.6 ± 13.6	$102.4 \pm 14.3 (5)$
$0.38 \pm 0.07\ddagger$	$13.2 \pm 2.9 (5)$	$0.59 \pm 0.13\ddagger$	$8.84 \pm 1.48 (7)$

this idea is the observation that repeated heel pricks in the newborn do not cause a rise in plasma GH levels (Milner and Wright, 1967; Stubbe and Wolf, 1971). Since two similar groups have been studied, valid comparisons can be made between them regarding the cause and effect of insulin secretion in erythroblastosis fetalis. The glucose in ACD transfusions caused a rise in plasma glucose and insulin similar to that reported previously (Milner and Wright, 1966). No significant change occurred in the plasma levels or balances of glucose and insulin in AC transfusions. These observations, when coupled with the finding that in ACD transfusions there was a significant negative balance of insulin and a significant positive balance of glucose, demonstrate that insulin secretion occurred in response to the glucose infusion. It is not known whether insulin stimulated the cellular uptake of glucose, as the volume of distribution of glucose is greater than the plasma space. The rise in plasma glucose during the transfusion did not necessarily indicate the distribution of glucose in other compartments.

In both types of exchange transfusion there was a similar negative FFA and glycerol balance. Glucose infusion had no effect on plasma glycerol levels which remained steady during the transfusion and the negative glycerol balance in each type of transfusion can be explained by the lower plasma glycerol levels in donor blood. Thus, as for GH, there was a net production of glycerol in both AC and ACD transfusions. For FFA, there was an important difference between ACD and AC transfusions. Despite the similar difference between the plasma FFA levels in the donor blood and the infant at the start of the transfusion, in ACD transfusions there was a significantly greater fall in plasma FFA than in infants receiving AC transfusions. It may be concluded that in ACD transfusions FFA disappeared from the infant's intravascular compartment in addition to being washed out of the infant. The inference is that net lipogenesis was stimulated by hyperglycaemia and secondary hyperinsulinaemia.

There is little information on glucagon secretion in the human newborn. Milner, Fekete, and Assan (1972) have shown that infants with erythroblastosis have higher plasma glucagon levels than normal infants. The levels were also higher than those in the donor blood, which was representative of adult peripheral venous blood collected postprandially. In the normal adult glucose infusion causes a fall in plasma glucagon levels (Unger *et al.*, 1970), and it was of interest to see if this occurred in the newborn. In both ACD and AC

transfusions there was a similar negative balance and a similar gradual fall in plasma glucagon levels during the transfusion. The findings could be explained by the difference in the plasma glucagon in the blood of the donor and infant at the start of the transfusion. The differences between ACD and AC transfusions were due to the fact that the mean difference between the donor and infant plasma glucagon was greater in the ACD than in the AC transfusions. It is possible to conclude that glucose infusion had no effect on glucagon secretion in the transfusions. However, it must be noted that the conditions of collection of blood samples for glucagon assay were not ideal (Heding, 1971). The possibility exists that glucagon was partially destroyed during collection and that larger changes in plasma levels and glucagon balance could have occurred during the transfusion than those actually recorded.

The concept of 'small-for-dates' is of doubtful validity when applied to infants with erythroblastosis fetalis. However, since differences in insulin secretion have been shown between normally grown and small-for-dates infants who are otherwise normal (Le Dune, 1972), it seemed preferable to subdivide the infants in the present study into 2 clinically defined subgroups. The fact that similar results were obtained in both NFD and SFD infants emphasizes the qualitative and quantitative validity of the results.

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