J. Physiol. (1938) 92, 459–466 612.647:615.361.37

THE EFFECT OF LARGE DOSES OF INSULIN ON THE FŒTAL SHEEP AND GOAT

By R. PASSMORE[1] and H. SCHLOSSMANN

(*From the Departments of Physiology and Pathology, Cambridge*)

(*Received* 21 *February* 1938)

Unlike most animals sheep and goats have a higher glucose level in the fœtal blood than in the maternal. It was of interest, therefore, to investigate how far the facts concerning the fœtal carbohydrate metabolism in dogs, which were discussed in a previous paper [Schlossmann, 1938], hold true for the fœtal lamb and goat.

Methods

Blood sugar was determined by Hagedorn & Jensen's method, and lactic acid by that of Friedemann, Cotonio and Shaffer.

The abdomen and uterus of the ewes and goats were opened and the fœtuses delivered into the saline bath at 37° as described previously [Schlossmann, 1932, 1938].

Results

Initial blood-sugar levels of mother and fœtus

Table I shows the initial blood-sugar levels of mother and fœtus. The fœtal are distinctly higher than the maternal levels in all cases except the last, where that of the mother was abnormally high.

It can be seen from the data collected by Needham [1931, Table 238] that in man, dog, rabbit, guinea-pig and rat the levels of the blood sugar are higher in the maternal than in the fœtal blood; whereas a higher level in the fœtal blood has only been found by Aron [1924] in the cow and pig. As a general rule then, the blood-sugar level is higher in the maternal blood, except in ungulates.

In previous papers [Schlossmann, 1930, 1932] the regulation of the passage of glucose from the mother to the fœtus was considered to be due to the difference in the sugar levels of the maternal and fœtal blood,

[1] Gwynaeth Pretty Student.

and a secretory activity of the placenta had been denied. The finding of the higher blood-sugar level in the fœtus of ungulates necessitates some other factor besides the sugar level in the maternal blood which determines the passage to the fœtus in these animals. We cannot at present give a satisfactory explanation of this mechanism which is peculiar to ungulates.

The differences in the blood-sugar levels of the umbilical artery and vein (Table I) are usually small. In Exp. 5 maternal blood was taken by puncturing the small arteries and veins which go to and come from the

TABLE I. Glucose content of the maternal and the fœtal blood in pregnant sheep and goats at the beginning of the experiments. All animals were near term. Figures in mg. per 100 ml. Intravenous anæsthesia with urethane (Exps. 1–5) or pernocton (Exps. 6 and 7).

		Mother		Fœtal blood sugar Blood taken from		
No.		Blood from	Blood sugar	Umbilical artery	Umbilical vein	Carotid artery
1	Sheep	Jugular vein	62	71	76	—
2	,,	,, ,,	88	90	98	—
3	,,	Carotid artery	112	—	—	188
4	,,	,, ,,	110	—	—	172
5	Goat	Uterine artery	105	160	156	—
		Uterine vein	85	—	—	—
6	,,	Jugular vein	105	184	184	—
7	,,	,, ,,	183	183	180	—

cotyledons. Here the difference in the blood-sugar levels between arterial and venous blood was 20 mg. p.c., and this difference remained constant over 3 hr. This shows that a comparatively large amount of sugar is taken up by the cotyledons. But the mechanism of the glucose passage through the placental barrier is made no clearer, as the amount and the glucose content of the blood inside the cotyledons is unknown. A part of this blood might be stagnant and more or less out of the normal circulation.

Effect of glucose infusion

The placenta in the dog is permeable to glucose [Schlossmann, 1932]. Considering the higher glucose level of the fœtal blood in sheep it was necessary to determine whether the same is true in these animals. 200 ml. of a 20 p.c. glucose solution were infused into the jugular vein of an ewe near term, the infusion lasting 3 min. The rapid rise in the maternal blood sugar was followed by a less sharp rise in the fœtal blood sugar (Fig. 1). After 25 min. the two curves crossed over, and there was a gradual fall to the normal level, the fœtal values being above the maternal. The result is exactly the same as that obtained previously in

dogs, and it is only consistent with the view that—at least under these experimental conditions—the placenta is permeable to glucose in both directions (Hoeber's "physikalische Permeabilität").

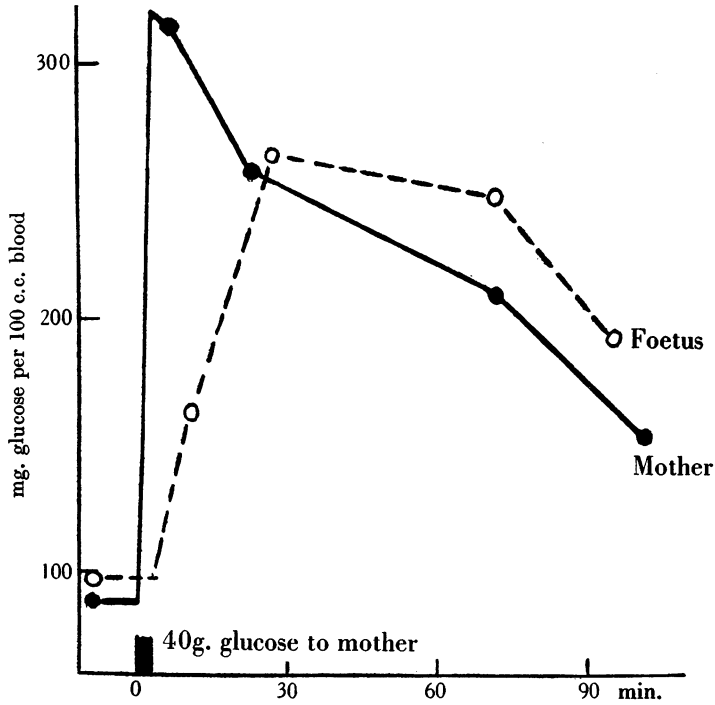

Fig. 1. Experiment 17. iii. 36. Sheep tupped 26. x. 35. Weight of the ewe 38·2 kg., of the fœtus 3·85 kg. Urethane anæsthesia intravenously. 200 ml. of 20 p.c. glucose infused to mother intravenously within 3 min. Maternal blood was taken from jugular vein, fœtal blood from umbilical vein.

Effect of insulin on fœtus and mother

After opening the uterus and delivering the fœtus into the saline bath, samples of blood were taken from the mother and the fœtus. Insulin was then given to the fœtus intravenously, and other samples of blood were taken after suitable intervals. In the first experiment 200 units of insulin were injected intraperitoneally into the fœtus without effect on the fœtal and maternal blood-sugar levels. The result of another experiment is shown in Table II. After the injection of 300 units of insulin the fœtal blood-sugar level dropped from 188 to 126 mg. p.c., and the lactic-acid level increased slowly. Shortly before death a small increase of the blood-sugar and a sudden rise of the lactic-acid level was

TABLE II. Experiment 3. iii. 37. Weight of the ewe 34·1 kg. Urethane intravenously. Fœtus 131 days old, weight 2·5 kg. Blood samples from maternal and fœtal carotid artery. 300 units of insulin (Hoechst) injected intravenously into the fœtus. Four hours after the injection the umbilical cord was tied and the lamb was removed from the saline bath. It failed to breathe and died after 15 min. The last blood sample was taken by heart puncture immediately after death. Values in mg. per 100 ml.

Insulin injection min.	Blood sugar		Lactic acid	
	Mother	Fœtus	Mother	Fœtus
10 before	112	188	11	15
90 after	117	126	15	29
180 „	85	138	11	45
240 „	84	128	10	49
255 „	—	142	—	99 Fœtus dead

found. In the experiments shown in Fig. 2 and Table III insulin was also given intravenously to the mother about 2 hr. after the fœtus had been injected, in order to observe the effect of insulin on the mother.

Despite the large doses of insulin the fall in the fœtal blood sugar was small in these experiments, and the fœtal glucose content was always above the maternal. After the injection of insulin the lactic-acid level of the fœtal blood rose steadily throughout the experiment. The differences of the blood-sugar and lactic-acid levels between umbilical vein and umbilical artery—marked in dogs after the injection of insulin—are insignificant in fœtal lambs. There is no such evident fœtal hypoglycæmia to compensate as in fœtal dogs [Schlossmann, 1938]. The rise of the lactic-acid level in the blood of fœtal lambs is probably mainly due to an increasing impediment to transfer of lactic acid through the placenta to the mother. Fœtal lambs and goats are much more resistant to insulin than fœtal dogs, the insulin doses being nearly the same in these experiments (60–415 units per kg.) as in those on dogs (65–235 units per kg.).

After tying the umbilical cord and removing the fœtus from the saline bath a sudden rise in the blood-sugar and lactic-acid levels was observed (Table II and Fig. 2). The rise in the lactic-acid level may be explained by the fact that after birth no more lactic acid can pass across the placental barrier to the mother. The rise in the blood sugar is more difficult to understand, considering the large amounts of insulin injected into the fœtus. Perhaps the mobilization of glycogen from the fœtal stores is due to the cooling after removal from the saline bath.

The lamb into which 1200 units of insulin had been injected was still alive 6 hr. after birth, and in no experiment were convulsions observed. The adult sheep, too, is comparatively resistant to insulin. Strand *et al.* [1934] injected 2–3 units of insulin per kg. of body weight into four

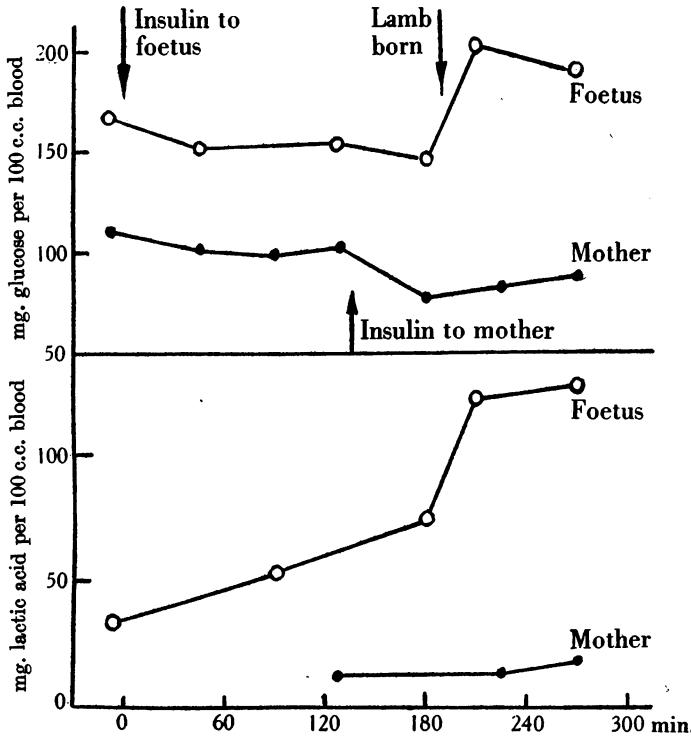

Fig. 2. Experiment 11. iii. 37. Weight of ewe 30·0 kg. Spinal anæsthesia with durocain, 2 hr. later 5 ml. dial intravenously. Weight of fœtus 3·18 kg., 139 days old. 1200 units of insulin (Hoechst) were injected intravenously into the fœtus, 135 min. later 150 units intravenously into the mother. 185 min. after the insulin injection into the fœtus the umbilical cord was tied and the lamb removed from the saline bath. Blood samples were taken from maternal and fœtal carotid artery. The maternal figures are represented by dots, the fœtal by circles.

TABLE III. Experiment 19. iii. 37. Weight of the goat 45·0 kg. Urethane intravenously. Fœtus 142 days old, weight 2·95 kg. Blood samples from uterine artery and umbilical artery. 600 units of insulin (Hoechst) were injected intravenously into the fœtus, and 2 hr. later 250 units intravenously into the mother.

Insulin injection into fœtus, min.	Blood sugar mg. per 100 ml. Mother	Fœtus	
10 before	105	160	
55 after	103	140	
120 „	99	140	Insulin to mother
180 „	101	163	
240 „	80		

normal sheep. The blood-sugar levels fell 20–30 mg. per 100 ml. within 45–90 min. No convulsions were seen, and the lactic-acid level of the blood was slightly higher only in one of the four animals. In our experiments on adult sheep and goats the intravenous injection of 5 units of insulin per kg. caused a drop in the blood-sugar level of 18–37 mg. per 100 ml. within 45–100 min. These figures are shown in Table IV together

TABLE IV. Effect of insulin on the blood-sugar level. Intravenous injection

	Weight kg.	Units injected	Units per kg.	Fall of blood sugar			Insulin sensitivity (fall of blood sugar per 5 units of insulin per kg.)
				From	To	mg. per 100 ml.	
Goat	45·0	250	5·5	99	80	19	17·0
Ewe	30·0	150	5·0	101	83	18	18·0
Ram	57·0	285	5·0	78	41	37	37·0
Lamb (24 hr.)	2·6	300	115·0	111	49	62	2·7
Fœtal sheep (131 days)	2·5	300	120·0	188	126	62	2·6
Fœtal sheep (139 days)	3·2	1200	415·0	172	148	24	0·3
Fœtal goat (142 days)	2·95	600	202·0	160	140	20	0·5

with those for the fœtal and newborn lamb and goats. The drop in the blood-sugar level caused by 5 units of insulin per kg. in the adult requires hundreds of units in the fœtus and the newborn. In order to get an indication of the relative sensitivity to insulin the maximum fall of blood sugar has been divided by each 5 units of insulin given per kg. of body weight. The figures (last column of Table IV) give no more than a rough idea, as they are based only upon the few experiments described above, and upon the observation that doses of 5 units per kg. have no effect on the blood sugar of the fœtal lamb.

Effect of fœtal lamb plasma on the action of insulin in the rabbit

It has long been known that certain cases of diabetes are peculiarly resistant to insulin. Recently de Wesselow & Griffiths [1936] have shown that the plasma from these diabetics, when injected into young rabbits, has an inhibitory effect on the normal action of insulin. They suggest that these patients have in their blood a substance with a diabetogenic action, possibly of pituitary origin, which antagonizes the action of insulin. We considered that the remarkable resistance of the fœtal lamb to insulin might be due to a similar substance.

To test this the response to an intravenous injection of 0·2 unit of insulin per kg. into rabbits weighing between 1 and 2 kg. was determined,

TABLE V. Rabbits fasting for 18 hr. Maximum observed fall in blood sugar as percentage of the initial blood-sugar level after intravenous injection of 0·2 unit of insulin per kg.

Rabbit	Insulin alone	Three hr. before 10 ml. horse serum	Three hr. before 10 ml. fœtal lamb plasma
1	58	—	32 (4 ml. only)
2	55	36	15
	52		
3	30	31	11
4	39	25	21

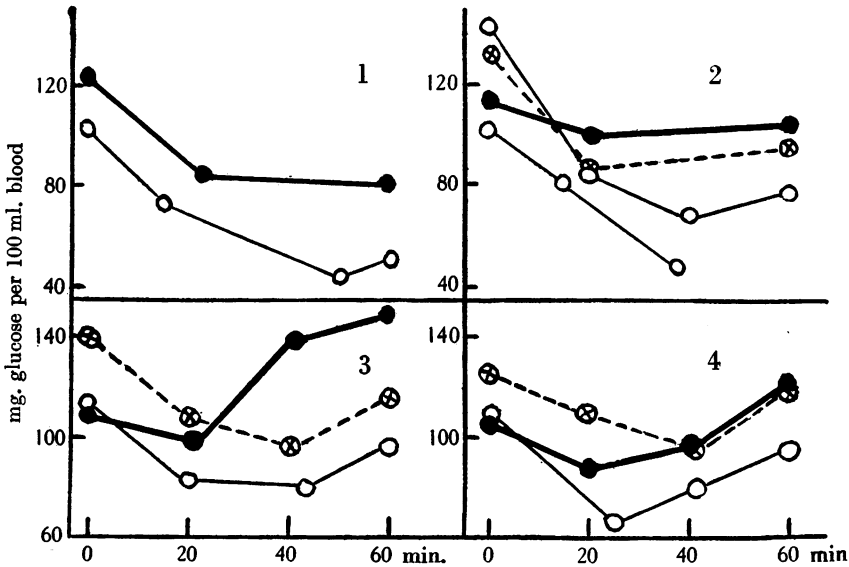

Fig. 3. Four rabbits, blood sugar in mg. p.c. ○——○ = 0·2 unit of insulin per kg. injected intravenously. ●——● = 10 ml. of fœtal lamb plasma (only 4 ml. in Exp. 1) injected subcutaneously, 3 hr. later, insulin as above. ⊗- - -⊗ = 10 ml. of normal horse serum injected subcutaneously, 3 hr. later, insulin as above.

according to the method of de Wesselow & Griffiths. A few days later the rabbits received subcutaneously 10 ml. of fœtal lamb plasma and 3 hr. later the standard dose of insulin intravenously. The insulin test was repeated a third time 3 hr. after 10 ml. of normal horse serum had been given subcutaneously as a control. Fig. 3 shows the results, and in Table V the maximum falls in the blood-sugar levels of all tests are collected. It will be seen that the fœtal lamb plasma always had a marked inhibitory effect, and in two of the four cases a small drop in the blood-sugar level was followed by an increase above the initial level. In two cases, however, horse serum had a similar though smaller effect.

Although these experiments are few, and not too well controlled, they indicate that the presence in the blood of a substance actively antagonistic to insulin might play a part in the resistance of the fœtal lamb to insulin.

SUMMARY

In the sheep and the goat the blood-sugar levels are substantially higher in the fœtus than in the mother. The permeability of the placenta to glucose is shown by infusing glucose into the mother.

The fœtal sheep and goat are scarcely affected by doses of insulin up to 415 units per kg. of body weight. An insulin-antagonistic substance seems to be present in the blood of fœtal lambs.

We wish to thank Sir Joseph Barcroft for his interest and his help in this work.

REFERENCES

Aron, M. (1924). *Arch. int. Physiol.* **22**, 273.
Needham, J. (1931). *Chemical Embryology.* Cambridge.
Schlossmann, H. (1930). *Z. ges. exp. Med.* **72**, 401.
Schlossmann, H. (1932). *Ergebn. Physiol.* **34**, 741.
Schlossmann, H. (1938). *J. Physiol.* **92**, 219.
Strand, R., Anderson, W. & Allcroft, W. M. (1934). *Biochem. J.* **28**, 642.
de Wesselow, O. L. V. & Griffiths, W. J. (1936). *Lancet*, No. 230, p. 991.