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REABSORPTION OF GLUCOSE, FRUCTOSE AND MESO-INOSITOL BY THE FOETAL AND POST-NATAL SHEEP KIDNEY

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The purpose of the present investigation was to study the excretion by the foetal kidney of the sheep of three normal components of foetal sheep blood, namely glucose, fructose and meso-inositol. Evidence has been put forward that in man these substances share some common element of the reabsorptive mechanism; thus Gammeltoft & Kjerulf-Jensen (1943) have suggested that glucose and fructose compete, while Daughaday & Larner (1954) suggested a common mechanism for glucose and meso-inositol.

The results reported here indicate that glucose reabsorption is well established in the foetus by the middle of gestation, whereas fructose and mesoinositol are only poorly reabsorbed.

METHODS

Studies were made on eight foetuses between 59 and 135 days gestation age. The foetuses were delivered by Caesarian section from Welsh ewes under procaine spinal anaesthesia, supplemented when necessary during the course of the experiment with intravenous thiopentone. The ewe was held in the supine position with the head and upper portion of the trunk elevated to an angle of about 45° above the horizontal. On delivery the foetus was placed on a heated platform fixed between the legs of the ewe. Care was taken to ensure that the cord was not under tension and that it was kept warm and moist. With older foetuses the head was initially delivered into a polythene bag containing 0·9 % NaCl solution, to prevent the onset of breathing. Tributaries of the umbilical veins and arteries were catheterized for intravenous administrations and blood sampling respectively. Exposure and cannulation of the foetal bladder was carried out in the manner previously described (Alexander, Nixon, Widdas & Wohlzogen, 1958).

Two lambs (10 hr and 7 days post-natal), were anaesthetized with intravenous thiopentone, the abdomen opened and the bladder cannulated directly. Catheters were inserted in the femoral vein and artery for administrations and blood sampling.

In two adult ewes experiments have been performed under spinal anaesthesia, in which the bladder was either cannulated directly or by the urethral insertion of a catheter. Experiments have also been performed on the unanaesthetized ewes at intervals over several weeks. With aseptic techniques, amethocaine (10–15 mg in 2·5 ml. of 4·5 % glucose) was injected into the subarachnoid space immediately above the sacrum. Long polythene catheters (Allen and Hanbury Ltd., No. 2) fitted with two-way taps were inserted into the dorsalis pedis artery and vein so that the tips lay in the large vessels. The catheters were filled with heparin saline solution (5000 i.u./ml.). After suturing the skin and applying iodine solution the leg was carefully wrapped in elastic bandages to include the catheters with their taps, which were held on the inner aspect of the thigh. The whole was finally held in place by a continuous wrapping of waterproof bandage. The bladder was catheterized by means of a self-retaining catheter (Foley, F. G. No. 16). Under these conditions the animals recovered from the spinal anaesthesia and could stand within 12 hr. The daily injection of heparin along the catheters ensured their patency. The animals remained free from infection. During the experiments the ewes were restrained by placing all four legs through holes in a canvas sling held on a framework at a height which just permitted the animal to stand. The animals became accustomed quite quickly to this restraint and would remain quiet for several hours.

Urine samples were collected over periods of 10 min, blood samples taken at the mid point of each collection period. The recorded values represent data derived from at least two such collection periods.

Shannon (1937–38) found that the plasma clearance of inulin is 97 % of the exogenous creatinine clearance in the sheep. In the present experiments inulin clearance was used as a measure of glomerular filtration rate in the ewes. Exogenous creatinine clearance was used in the lambs and in the foetuses, where endogenous fructose interferes with the inulin estimation. Creatinine or inulin alone or with glucose added were given intravenously in priming quantities, assessed on the basis of calculated body weight. These were followed by an infusion (a Palmer's continuous slow injector being used) to maintain the established plasma levels. In foetuses this was a 0·03–0·13 % solution of creatinine in 0·9 % NaCl or in approximately 2·5 % glucose (except in one case where 10 % glucose was used), given at 0·17 or 0·34 ml./min. In lambs a 0·13 % creatinine solution in 0·9 % NaCl or in 4·5 % glucose was infused at 0·68 ml./min. Adults received 1 % inulin in 0·6 % NaCl or 4·5 % glucose infused at 3·0–8·0 ml./min.

Creatinine was estimated by the method of King (1946); inulin was determined as fructose by the method of Chinard, Danesino. Hartmann, Huggett, Paul & Reynolds (1956). The urine and plasma samples (after deproteinization with NaOH and $ZnSO_4$) were analysed for glucose by the method of Huggett & Nixon (1957), fructose by the method of Chinard *et al.* (1956) and meso-inositol by the method of Campling & Nixon (1954).

RESULTS

Glucose excretion at existing plasma concentrations

The resting foetal plasma glucose concentration was variable but did not exceed 36 mg/100 ml. The excretion of glucose was zero in three of the foetuses and in all the new-born and adult animals. Glucose was excreted in the urine of the three remaining foetuses at rates ranging between 0·002 and 0·055 mg/min. The values obtained (Table 1) suggest that the foetal kidney acquires the capacity to reabsorb most of the filtered glucose before the mid point of gestation. The absence of reabsorption in the 59-day foetus may reflect inaccuracy in calculations involving very low urine flows.

The plasma fructose concentration was considerably greater than that of glucose. However, the rate of fructose reabsorption was not much more than that of glucose and resulted in a high rate of fructose excretion. In the one foetus examined, where the plasma inositol concentration was within the normal range, the rate of reabsorption was 0·79 mg/min.

TABLE 1. Mean urine flow, glomerular filtration rate, excretion and reabsorption rates of glucose and fructose in foetal, new-born and adult sheep under basal conditions

					Glucose			Fructose	
	Age (days)	Urine flow (ml./min)	G.F.R. (ml./min)	Plasma glucose (mg/100 ml.)	Excretion (mg/min)	Reabsorption (mg/min)	Plasma fructose (mg/100 ml.)	Excretion (mg/min)	Reabsorption (mg/min)
Foetal	59	0·05	0·12	33	0·039	0	160	0·12	0·07
	60	0·04	0·12	22	0	0·03	142	0·08	0·09
	77	0·12	0·47	9	0	0·04	88	0·31	0·10
	127	0·20	1·4	0?	0	0	43	0·20	0·40
	132 *	0·74	3·3	20	0·030	0·63	80	1·80	0·80
	135	0·87	2·5	36	0·055	0·87	132	2·12	1·26
Post-natal	0·5	0·08	10·3	11	0·002	1·30	—	—	—
	7	0·27	3·8	9	0	0·35	—	—	—
Adult	*A*	1·25	97·0	48	0	45·8	—	—	—
	B	2·1	117·0	71	0	83·0	—	—	—

* In the 132-day foetus (twin F_1) the mean plasma inositol was 34 mg/100 ml., the rate of excretion 0·35 mg/min and reabsorption 0·79 mg/min.

Glucose excretion with raised plasma glucose concentrations

Experimental elevation of the plasma glucose concentration resulted in the appearance of glucose in the urine in each case. The calculated rate of glucose reabsorption was also increased (Table 2).

In two foetuses where more than one plasma glucose level was attained the rate of reabsorption increased with the plasma glucose concentration. Only in the 77-day foetus were identical values for glucose reabsorption obtained for two different plasma glucose concentrations. These represent a Tm (tubular maximum) value of 1·2 mg/min. In the ewes *A* and *B* the mean Tm glucose was calculated to be 150 and 157 mg/min respectively.

Effect of phlorrhizin

Foetuses at 70, 77 and 132 days and the 10 hr lamb received phlorrhizin 8–22 mg/100 g body weight during the glucose infusion. In each case this resulted in plasma glucose clearances approximating the glomerular filtration rates. The response of the 77-day foetus to phlorrhizin administered during the glucose infusion is illustrated in Fig. 1. Calculated rates of tubular reabsorption before and after phlorrhizin are shown in Table 3. A considerable reduction in the rate of glucose transfer is shown in every case. However, the available data for the effect of phlorrhizin on fructose and inositol transfer are inconclusive.

TABLE 2. Mean urine flow, glomerular filtration rates, excretion and reabsorption of glucose, fructose and meso-inositol in foetal, new-born and adult sheep after elevation of the blood glucose concentration

	Age (days)	Body wt. (kg)	Urine flow (ml./min)	G.F.R. (ml./min)	Plasma glucose (mg/100 ml.)	Glucose Excretion (mg/min)	Glucose Reabsorption (mg/min)	Fructose Excretion (mg/min)	Fructose Reabsorption (mg/min)	Inositol Excretion (mg/min)	Inositol Reabsorption (mg/min)
Foetal	60	0·05	0·01	0·02	1540	0·18	0·15	0·03	0·01	—	—
	70	0·11	0·10	0·24	120	0·04	0·25	0·18	0·07	0·02	0·05
	77	0·19	0·06	0·43	125	0·02	0·52	0·21	0·15	—	—
			0·15	0·55	285	0·34	1·2	0·27	0·19	—	—
			0·14	0·43	410	0·53	1·2	0·24	0·12	—	—
	127	0·99	0·38	1·4	250	0·66	2·8	0·45	0·35	—	—
			0·28	1·3	490	2·78	3·4	0·69	0·38	—	—
			0·47	1·2	2000	11·7	13·0	1·03	0·05	—	—
Twins	132 F_1	2·34	0·88	3·9	78	0·03	3·1	2·10	1·2	0·42	0·93
	F_2	2·45	0·36	4·1	70	0·01	2·9	2·68	2·0	0·19	1·2
Post-natal	0·5	2·5	0·51	11·6	530	29·0	32·0	—	—	—	—
			0·38	9·2	510	22·4	24·0	—	—	—	—
			0·35	9·5	480	22·2	24·0	—	—	—	—
	7·0	1·94	0·29	2·9	840	9·7	14·0	—	—	—	—
			0·23	2·6	760	9·0	11·0	—	—	—	—
			0·24	2·7	690	10·0	10·0	—	—	—	—
Adult	*A*	32·4	13·0*	86·0	630	400·0	160·0	—	—	—	—
			7·5*	80·0	410	176·0	150·0	—	—	0·18	0·15
			8·8†	73·0	510	229·0	140·0	—	—	0·34	0·05
	B	29·4	1·6*	71·0	410	99·0	180·0	—	—	0·38	0·24
			15·0*	103·0	300	148·0	170·0	—	—	—	—
			6·5†	56·0	540	186·0	120·0	—	—	0·32	0·16

* Denotes animal conscious and standing. † Denotes animal under spinal anaesthesia.

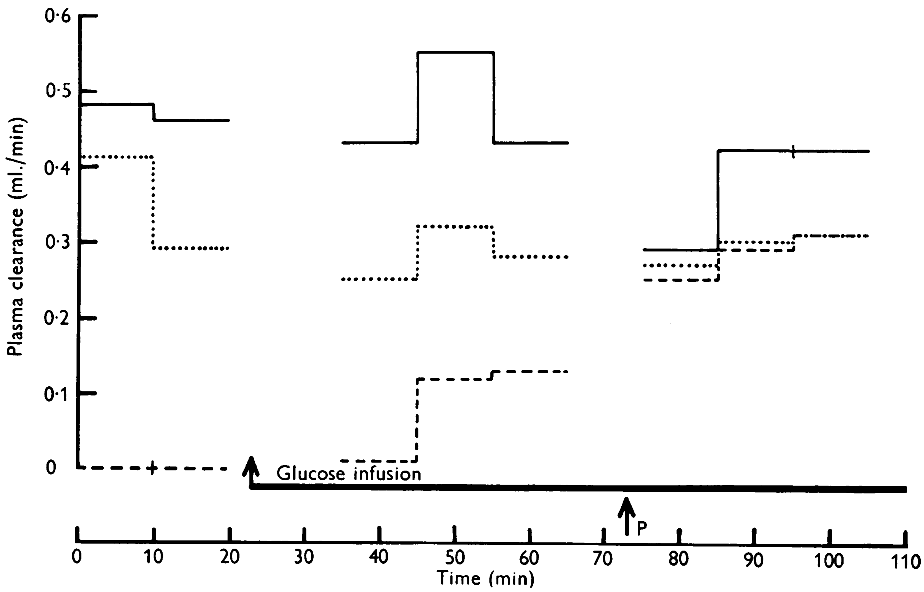

Fig. 1. Foetal age 77 days. Effect of phlorrhizin (20 mg I.V. at P) on the foetal plasma clearance of glucose - - -, fructose . . . , creatinine —— during a continuous glucose infusion to the foetus

TABLE 3. Mean reabsorption rates of glucose, fructose and meso-inositol before and after administration of phlorrhizin. Plasma glucose concentration elevated by a maintained continuous infusion

	Age (days)	Glucose reabsorption (mg/min) Before phlorrhizin	After phlorrhizin	Fructose reabsorption (mg/min) Before phlorrhizin	After phlorrhizin	Inositol reabsorption (mg/min) Before phlorrhizin	After phlorrhizin
Foetal	70	0·26	0·03	0·07	0·04	0·05	0·02
	77	1·2	0·47	0·12	0·08	—	—
	132	2·9	0·40	2·0	2·08	1·2	0·97
Post-natal	0·5	24·0	5·95	—	—	—	—

DISCUSSION

The results show that some reabsorption of glucose and fructose is occurring in the foetus under basal conditions. Indeed, all the filtered glucose is reabsorbed in many of the animals. Evidence in support of the early occurrence of an active reabsorption is provided by the demonstration of alkaline phosphatase activity in the proximal tubules of the foetal sheep metanephros as early as 43 days gestation age (Alexander, unpublished). Recently, Perry & Stanier (1962) have observed the absence of glucose in urine produced by the mesonephros of the foetal pig.

Elevation of the plasma glucose results in a considerable increase in the amount of glucose reabsorbed. This increase in glucose reabsorption may represent either an active reabsorptive process which has not been saturated or an active process which has indeed been saturated but where the further increase in glucose reabsorption represents merely diffusion down a concentration gradient across the tubular walls. The observed reduction in glucose reabsorption in the presence of phlorrhizin may be considered as interfering with either of these processes.

In view of the diversity of plasma glucose concentrations observed in relation to foetal age it was not possible to express the results in terms of tubular maximal transfers. However, in the 77-day foetus, where similar transfer rates were observed at two different plasma concentrations, the Tm value was calculated to be 1·2 mg/min or 6·2 mg/min/kg body weight. This compares with the adult value of 5·0 mg/min/kg body weight.

The results indicate that conservation of plasma glucose is effected *in utero*, at least in part, by an active tubular reabsorbing mechanism which is well established before the foetus enters the rapid phase of growth at about 90 days. It would appear also that a considerable margin of glucose reabsorptive capacity exists in foetal life.

SUMMARY

1. The urine of foetal sheep in the latter half of gestation is essentially devoid of glucose. Fructose and inositol occur in the urine but in amounts indicating some measure of reabsorption.

2. Elevation of the plasma glucose concentration revealed a considerable reserve in the capacity of the renal tubules to reabsorb glucose. The effect upon fructose and inositol reabsorption was variable.

3. Phlorrhizin decreased the rate of glucose reabsorption, but had little effect on the reabsorption of fructose and inositol.

4. A Tm glucose value as great as that of the adult was present in a 77-day foetus, when compared on the basis of body weight.

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