Gluconeogenesis in the Kidney-Cortex Slices of Normal Fed and Starved Sheep

Gluconeogenesis in the Kidney–Cortex Slices of Normal Fed and Starved Sheep

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1. The rate of gluconeogenesis from various substrates with the kidney-cortex slices of normal fed and starved sheep have been investigated.

2. Among short-chain fatty acids, propionate exhibited a very high rate of gluconeogenesis, but acetate and butyrate did not give any glucose formation in either group of sheep.

3. Of four glucogenic amino acids only glutamate yielded a high rate of gluconeogenesis and the rates were unexpectedly low or even negligible with asparate, alanine and serine. During the starvation, glucose formations from alanine and serine increased slightly, whereas that of glutamate rather decreased.

4. Most of the seven organic acids yielded glucose and glycogen at a high rate, particularly fumarate and succinate were very effective precursor. The study of rat-kidney-cortex slice incubated under comparable conditions showed that the rates of gluconeogenesis from lactate and oxaloacetate in sheep-kidney were remarkably lower than those in rat-kidney.

5. Starvation for 2 weeks decreased the rates of gluconeogenesis from most precursors. It was probably connected with the relatively long duration of the starvation period.

Ruminants present in their carbohydrate metabolism certain peculiarities which distinguish them from monogastric animals. Ruminants derive a large part of their supply of metabolic substances from the end products such as volatile fatty acids produced in rumen fermentation. Also ruminants absorb very little glucose from their digestive tracts and depend almost entirely on gluconeogenesis for their glucose supply.

Recent publications have shown that relatively large quantities of glucose are synthesized by the ruminant and that these quantities are similar to those in postabsorptive monogastric animals when they are compared on a metabolic body weight basis (1). Therefore it is well established that the synthesis of glucose from nonhexose sources has a special significance in ruminant nutrition.

In this regard, recent works using the isotope dilution techniques in ruminants have indicated that the substrates for gluconeogenesis are available as

propionate and amino acids from the digestive tract, glycerol from triglyceride breakdown, lactate from muscle glycolysis and amino acids from protein turnover (2). However, the entry of glycerol (3) and free fatty acids (4) are low in feeding animals. Thus, it is recognized that propionate and amino acids are the main sources of glucose in feeding ruminants (5, 6, 7).

In the mammalian animals, gluconeogenesis is known to occur not only in the liver but also in the kidney cortex (8, 9, 10). It is believed that the total gluconeogenic capacity of the liver is much greater than that of the kidney-cortex because of the large mass of the liver and the availability of substrates in portal circulation. However it was observed that per unit of weight the glucogenic capacity of the kidney-cortex is somewhat higher than that of the liver (11). Furthermore, as an experimental material for the study of gluconeogenesis, that the kidney offers major advantages over the liver was pointed out by Krebs and others (10). Because of a higher content of preformed carbohydrates in the liver than in the kidney, it is difficult to measure the net carbohydrate increases which occur in the liver after incubation with precursors. In contrast the increment of carbohydrate in the kidney on incubation is relatively large. In addition, kidney slices appear to be more permeable to certain substrates, in particular polyvalent anions.

Although the in vitro study of gluconeogenesis in ruminants was done with isolated perfused goat liver (12) and liver slices (13), no work has been done on the renal gluconeogenesis.

In the present paper, the rates of gluconeogenesis from various substrates which is known to be glucogenic substrates was investigated in the kidney-cortex slices of normal fed and starved sheep.

Experimental and Methods

Experimental animals

Five adult ewes weighing approximate 40 kg. were used for this study. Of them two ewes were used for the normal fed group in which 500 g. of orchard grass hay and 500 g. of commercial formula concentrates (wheat bran, corn, soybean meal, molasses and minerals; TDN, 68%; DCP, 14.5%) were fed once a day. The other three ewes were used for a starved group. They were given only water for 2 weeks and lost body weight by 15 to 18% during the starvation period.

Treatment of tissue

The sheep were exsanguinated from bilateral carotid artery. The kidneys were immediately removed, rinsed with a cold solution of 1.15% KCl and the cortex isolated from the medulla. The cortexes were sliced free hand by the method of Deutsh (14). Five hundred milligrams of the slices were placed in a polyethylene test tube containing 3 ml of Krebs Ringer bicarbonate buffer or Krebs Ringer phosphate buffer, pH 7.4 and shaken for 30 min. at 37°C with air in the gas phase.
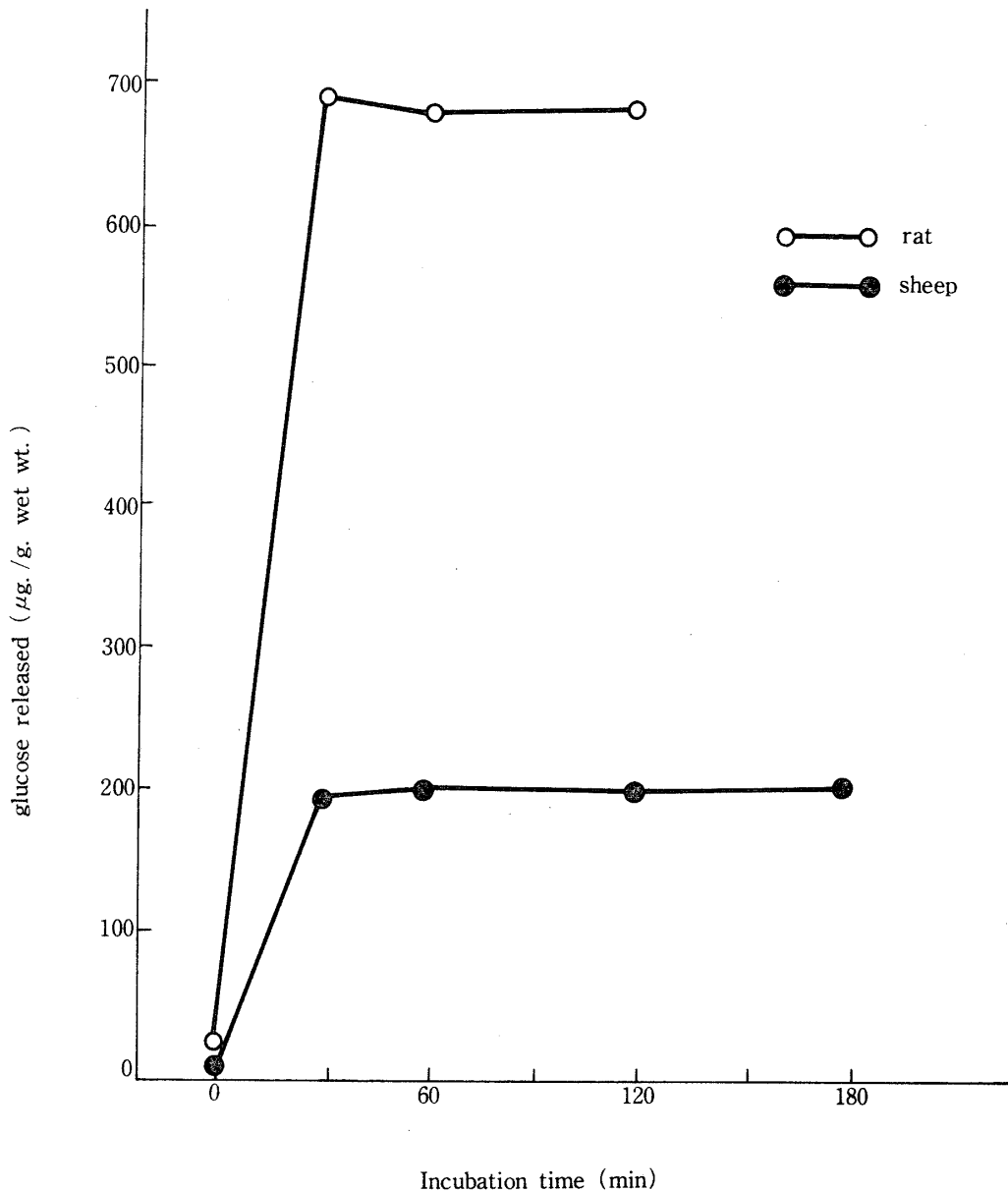
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Fig. 1. Time-course of release of glucose in incubating medium from kidney-cortex slices
  of sheep and rat.
Five hundred milligrams (wet wt.) of slices were incubated in Krebs-Ringer phosphate
(3 ml.) without any substrate at 37°C. Gas phase, 100% O_2.

This treatment was carried out to obtain accurate measurement for the net
glucose formation, which occur after the incubation with precursors, by reducing
the preformed glucose in slices during the preincubation. As shown in Figure 1,
the amount of glucose released in the medium was maximum during first 30 min.
of incubation and leveled off thereafter. In accordance with this result, kidney
slices were preincubated for 30 min. in this study. At the end of the preincubation,
the slices were immediately removed, blotted on filter paper and divided in half.
Each half was accurately weighed at 200 mg. Half of the slices were placed in a

polyethylen test tube containing 3.3 ml of KRB or KRP buffer and 0.01 M of the substrate. The other half of the slices were placed in a polyethylen test tube containing 3.3 ml of KRB or KRP buffer without the substrate. All polyethylen tubes were incubated for 2 hr. at 37°C at a shaking rate of 120 ossillation per min. with O_2+CO_2 (95: 5) in KRB buffer or O_2 in KRP buffer as gas phase. The reaction in the mediums were stopped by the addition of 0.5 ml of 40% trichloroacetic acid. The slices in the medium were homogenized throughly with a glass rode and centrifuged at 4000 r.p.m. for 15 min.

The substrates used are volatile fatty acids such as acetate, propionate and butyrate, four amino acids such as L-glutamate, L-aspartate, L-alanine and L-serine, and intermediates of the tricarboxylic acid cycle such as pyruvate, L-lactate, citrate, α-ketoglutarate, oxalocetate, succinate and fumarate. The glucose and glycogen contents of liver and kidney from each sheep were also immediately determined.

Analytical Methods

Assay of glucose

Glucose was determined by the glucose oxidase method as described by Hugget & Nixon (15) with a slight modification. After the deproteinization of the sample, 2 ml of the supernatant were mixed in a test tube with 5 ml of the glucose oxidase reagent containing glucose oxidase, peroxidase (Sigma Chemical Co.) and o-dianisidin (Merck), and incubated for 1 hr. at 37°C. After incubation, the extinction of the sample was mearsured spectrophotometrically at 440 mμ.

Assay of glycogen

One ml of deproteinized supernatant was hydrolyzed with 1 ml of $2N-H_2SO_4$ in a boiling water bath for 2.5 hr. Then the glucose content was determined by the glucose oxidase method. The glycogen content was obtained by subtracting the value of glucose determined before the hydrolysis from the total glucose value after the hydrolysis.

Results

Glucose and glycogen contents of kidney and liver in normal fed and starved sheep

As shown in Table 1, in normal fed sheep, the glucose content of kidney-cortex was found to be 0.0495g/100g. wet wt. and correspond to about 1/10 of liver glucose. Also the glycogen content of kidney-cortex was found to be 0.0494g/100g. wet wt. and correspond to about 1/60 of that in liver. The glucose and glycogen contents in kidney-cortex were almost the same, whereas liver glycogen content was about four fold the glucose content. In starved sheep, the glucose and glycogen contents of kidney-cortex did not differ from that of normal fed sheep. However, the liver glycogen content decreased to about a half of that of normal fed sheep, although liver glucose content somewhat decreased during a starvation

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TABLE 1.   *Glucose and Glycogen Contents in Kidney and Liver of Sheep*

Tissue	Condition of sheep	Glucose (g/100 g wet wt.)	Glycogen (g/100 g wet wt.)
Kidney	Fed	0.0495±0.0120 (3)	0.0494±0.0088 (3)
	Starved	0.0439±0.0141 (3)	0.0426±0.0026 (3)
Liver	Fed	0.66 (2)	2.72 ±0.54 (3)
	Starved	0.50 (2)	1.14 ±0.09 (3)

Each result is given as means±S.D. with the numbers of determinations in parentheses.
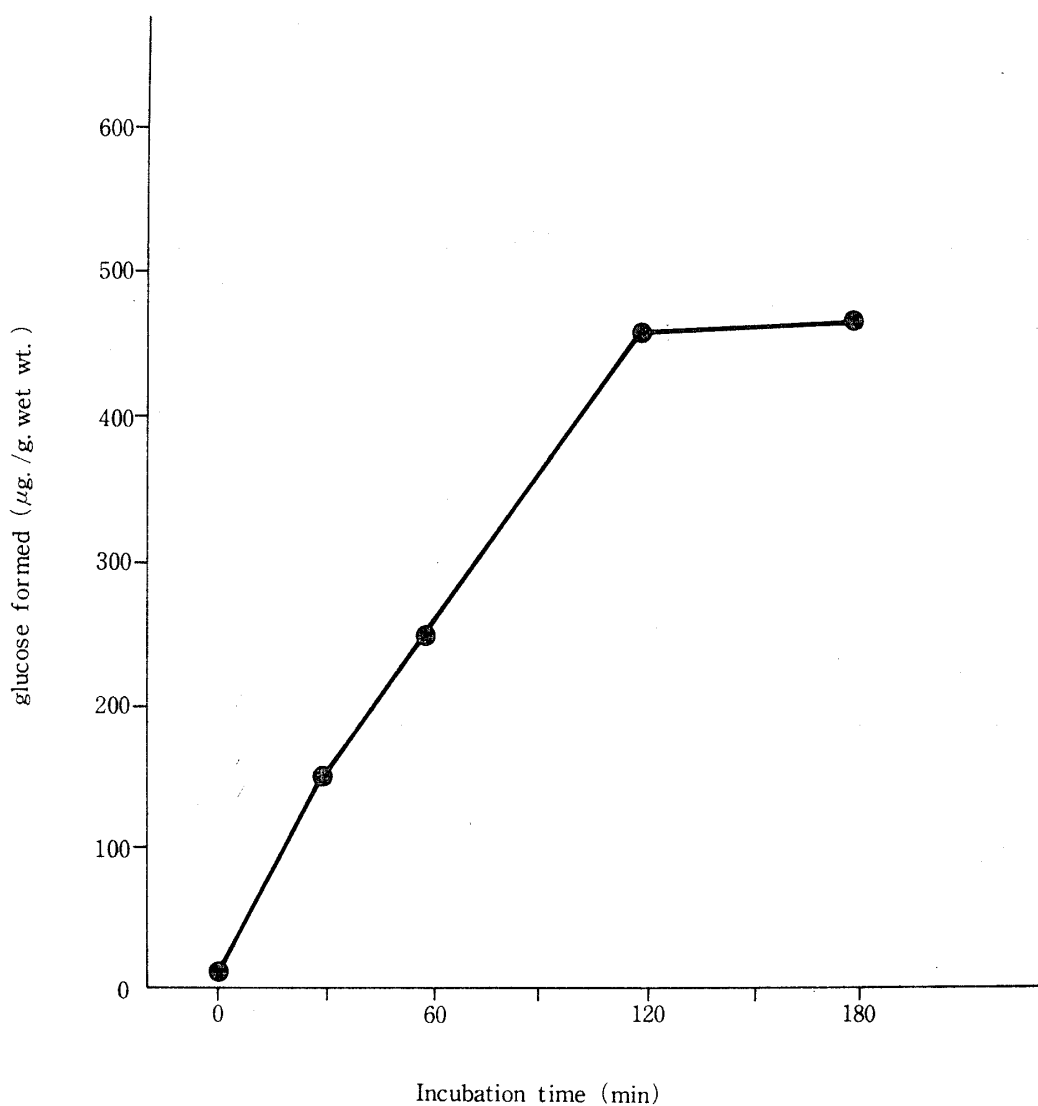

Incubation time (min)

Fig. 2.   Time-course of glucose formation by sheep kidney-cortex slices incubated with propionate.

After the preincubation for 30 min., 200 mg. (wet wt.) of slices were incubated in Krebs-Ringer bicarbonate (3.3 ml.) with 0.01M propionate at 37°C. Gas phase, O_2+CO_2 (95:5).

of 2 weeks.

Time-course of glucose formation

To what extent glucose formation from the precursor in kidney-cortex slices develops in relation to the proceeding of the incubation time was examined.

After the preincubation of 30 min., kidney-cortex slices of normal fed sheep was incubated for 3 hr. with propionate, which is known to be a effective precursor for gluconeogenesis. During the first 2 hr. the glucose formation developed steadily and then the rate decreased (Fig. 2). On the basis of this data, the incubation of 2 hours duration was carried out in this *in vitro* study.

Glucose and glycogen formation from short-chain fatty acids

As shown in Table 2, in both normal fed and starved sheep, propionate gave a high rate of glucose formation, but the acetate and butyrate yielded negligible amounts of glucose. In normal fed sheep, the quantity of glucose formed from propionate indicated that 28 per cent of the added propionate was converted into glucose. By starvation the rate of gluconeogenesis from propionate was reduced to 40 per cent of that in normal fed sheep. The rate of glycogen formation from propionate in starved sheep was 32 μg./g. wet wt. more than that of normal fed sheep. Nevertheless, the quantity of glycogen formed was only 5 to 20 per cent of that of the glucose formed. Glycogen formations from acetate and butyrate were not detectable in the kidney slices of either group.

Glucose and glycogen formation from amino acids

As shown in Table 3, in both normal fed and starved sheep the rate of

TABLE 2. *Formation of Glucose and Glycogen from Short-Chain Fatty Acids in Kidney-Cortex Slices of Fed and Starved Sheep*

Substrate	Glucose formed (μg./g.wet wt.)	
	Fed	Starved
Acetate	0	0
Propionate	925	399
Butyrate	0	10

Substrate	Glycogen formed (μg./g.wet wt.)	
	Fed	Starved
Acetate	0	0
Propionate	50	82
Butyrate	0	0

After the preincubation for 30 min., 200 mg. (wet wt.) of slices were incubated in Krebs-Ringer phosphate with acetate and butyrate, or in Krebs-Ringer bicarbonate with propionate for 2 hrs. at 37°C. The substrate concentration was 0.01 M.

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TABLE 3. *Formation of Glucose and Glycogen from Amino Acids in Kidney-Cortex Slices of Fed and Starved Sheep*

Substrate	Glucose formed (μg./g. wet wt.)	
	Fed	Starved
L-Glutamate	307.5	290.0
L-Aspartate	2.5	0
L-Alanine	0	42.5
L-Serine	0	70.0

Substrate	Glycogen formed (μg./g.wet wt.)	
	Fed	Starved
L-Glutamate	77.5	65.0
L-Aspartate	0	0
L-Alanine	10.0	6.5
L-Serine	0	15.0

After the preincubation for 30 min., 200 mg. (wet wt.) of slices were incubated in Krebs-Ringer phosphate with each substrate for 2 hrs. at 37 C. The substrate concentration was 0.01 M.

TABLE 4. *Formation of Glucose and Glycogen from Various Organic Acids in Kidney-Cortex Slices of Fed and Starved Sheep*

Substrate	Glucose formed (μg./g.wet wt.)	
	Fed	Starved
Pyruvate	149.0	7.25
L-Lactate	165.0	67.5
Citrate	233.5	86.0
a-Ketoglutarate	195.0	131.0
Oxaloacetate	57.5	34.5
Fumarate	955.0	760.0
Succinate	625.0	357.5

Substrate	Glycogen formed (μg./g.wet wt.)	
	Fed	Starved
Pyruvate	3.5	192.5
L-Lactate	85.0	22.5
Citrate	75.0	74.0
a-Ketoglutarate	180.0	89.0
Oxaloacetate	11.8	30.0
Fumarate	45.0	400.0
Succinate	100.0	52.5

After the preincubation for 30 min., 200 mg. (wet wt.) of slices were incubated in Krebs-Ringer phosphate with each substrate for 2 hrs. at 37°C. The substrate concentration was 0.01 M.

gluconeogenesis from glutamate was maximum among the four amino acids. Although glucose formation from alanine and serine were not evident in normal fed sheep, considerable amounts of glucose was formed from both substrates in

starved sheep. No glucose or glycogen formation from aspartate were observed in any of the slice.

Glucose and glycogen formation from organic acids

In both slices of normal fed and starved sheep, all of the intermediates of carboxylic acid cycle yielded a considerable rates of gluconeogenesis (Table 4). Of the organic acids used, fumarate was the most effective precursor for gluconeogenesis and succinate was the next. By starvation of 2 weeks duration, the rates of glucose formation from pyruvate, lactate, succinate and citrate decreased from 1/2 to 1/3 of that in normal fed sheep. Unlike those organic acids, glucose formations of fumarate and α-ketoglutarate were not affected seriously by starvation. Contrary to glucose formation, the rates of glycogen formation from fumarate and pyruvate in the starved sheep were about 9-fold and about 60-fold respectively of that in normal fed sheep. However glycogen formation from lactate and succinate decreased and those from citrate and α-ketoglutarate were not affected by starvation.

The study of rat kidney slice incubated under comparable conditions showed that the rate of glucose formation from lactate in rat kidney (555 μg./g. wet wt.) was much greater than that in sheep. Also the quantity of glucose formed from oxaloacetate in rat kidney was 1409 μg./g.

Discussion

Krebs and others (10) reported that kidney of well-fed rats contains 0.05 per cent glucose and 0.01 per cent glycogen. As presented in this paper, the content of glucose and glycogen in kidney from normal fed sheep are almost the same (0.05 per cent) and were not affected by starvation of 2 weeks duration. The corresponding values for fed sheep liver were 0.66 per cent glucose and 2.72 per cent glycogen. These values may suggest that kidney is a more suitable material, rather than liver, for the measurement of the net glucose formation after incubation with various precursors. In fact, the results obtained indicate that sheep-kidney-cortex slice formed glucose at a considerable rate from most substrates, as compared with those without substrate.

Among the short-chain fatty acids which are the important energy source for ruminants, propionate was a very effective precursor for gluconeogenesis in kidney-cortex from both fed and starved sheep. However no glucose was formed from acetate or butyrate. Krebs and Yoshida, who studied glycogenesis in kidney slices from 13 different species, observed that the rates of glucose formation from various substrates considerably varied with different species and that propionate was highly effective in sheep and rabbit but less so in cattle and guinea pig kidney (16). The hyperglycemic effect after butyrate administration was observed in sheep and goat (17, 18), and the net conversion of large amounts of butyrate into

carbohydrate by the perfused goat liver (12) has been reported by several investigators. However Black and others, from the *in vivo* study using lactating cows (19), and Leng and Annison, from the *in vitro* study using sheep liver slices (13), demonstrated convincingly the absence of metabolic pathways that would account for the glycogenic behaviour of butyrate. The results presented here were in agreement with their observations.

Of four amino acids examined, glutamate formed glucose at the highest rate in both kidney slices from fed and starved sheep. Whereas aspartate, alanine and serine, which are known to be glucogenic precursors in rat liver slices, formed only small amounts of glucose in kidney slice from fed sheep. Takahashi and others, in a study of the gluconeogenesis in sheep kidney-cortex slices using ^{14}C-labelled substrates, observed that much more than 90 per cent of total ^{14}C activities of aspartate added in the incubation medium was found in $^{14}CO_2$ (20). This observation suggests that amino acids are not only converted to glucose but are also readily oxidized under some physiological conditions. Thus the low rate of glucose formation from aspartate and others, as presented here, may be related to this suggestion. In fact, the rates of glyconeogenesis from serine and alanine were much higher in kidney of starved sheep than that of fed sheep.

Various organic acids including intermediates of tricarboxylic acid cycle yielded glucose in much higher rates in comparison with those from amino acids in both slices from fed and starved sheep, particularly very high rates of glucose formation were obtained with fumarate and succinate. Krebs and others observed that lactate, pyruvate, succinate, oxaloacetate and oxoglutarate showed marked gluconeogenesis in rat-kidney-cortex slices (10). The result observed in sheep kidney was similar to that observed in rat kidney with the exception of oxaloacetate. In fed sheep kidney slice, the rate of glucose formation from oxaloacetate was considerably lower than those from other organic acids and its rate was only 3 per cent of those of rat kidney slice under comparable conditions of incubation. In this regard, Muramatsu (personal communication) observed that unlike rat liver mitochondria, the conversion of oxaloacetate to phosphoenolpyruvate dose not occur in sheep liver mitochondrial fraction unless the mitochondrial membrane is destroyed. If such a difference on permeability of liver mitochondrial membrane to oxaloacetate exists between different species in kidney-cortex also, it may be related to the relatively low rate of glucose formation from oxaloacetate in sheep kidney slices.

With the exception of alanine and serine, the rates of glucose formation from most added substrates significantly decreased by starvation. It has been observed that several enzymes involved in gluconeogenesis, e.g. glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, increase their activities during starvation (21). Krebs and others showed that the rates of gluconeogenesis from various precursors in rat kidney slices increased significantly by starvation for 48 hours and by feeding

on a low carbohydrate diet (10). These differences may be due to the relatively long duration of starvation (2 weeks) in this study, as suggested by the following observation: a few small particles of calciumlike substance were found in the kidney tissue of starved sheep. It is probable that the long period of stavation may have depressed the renal function which resulted in the low gluconeogenic capacity.

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