Gluconeogenesis in a carnivorous bird (black vulture)

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Although numerous papers have been published on different aspects of carbohydrate and fat metabolism of granivorous birds, relatively few studies have been performed in carnivorous birds. This is surprising in view of the fact, known for a long time, that pancreatectomy induces permanent diabetes in carnivorous, but not in granivorous birds (16), suggesting marked differences in the metabolic patterns of the two groups of animals. Recently, it has been shown (15) that diabetes following total pancreatectomy also occurs in the black vulture (Coragyps atratus), a carnivorous bird which feeds on the carcasses of dead animals. Since the feeding habits of these animals lead them to ingest large amounts of protein and relatively little carbohydrate, glucose synthesis from noncarbohydrate sources probably is a prominent feature of their intermediary metabolism. For this reason, it was thought of interest to investigate the gluconeogenic capacity of the liver in the black vulture. In the present experiments, in addition to determining the rate of glucose synthesis from alanine by liver slices, in vitro, the activities of three "key" enzymes of gluconeogenesis: glucose-6-phosphatase (EC 3.1.3.1), fructose-1,6-diphosphatase (EC 3.1.3.5), and phosphoenolpyruvate carboxykinase (EC 6.1.1.2), were measured, since preliminary experiments showed that the black vulture (Coragyps atratus), a carnivorous bird (black vulture) which feeds on the carcasses of dead animals, may be able to synthesize glucose and its precursors from dietary protein, and that this capacity is probably related to the high protein content of the food.

Animals

Male black vultures (Coragyps atratus) were utilized 15-60 days after capture. During this period their food consisted of carcasses of dogs. The birds did not lose weight in captivity and weighed between 1.6 and 2.6 kg when obtained. Male crossbred chickens (Indian River-Cornish × Plymouth Rock) weighing between 1.4 and 2.4 kg, were maintained on a grain commercial diet (65% carbohydrate, 35% protein) for growing chicks. The birds were housed in groups in large cages and had access to food and water at all times. Fasted animals were given only water. All birds were sacrificed by decapitation.

Gluconeogenesis

The rate of gluconeogenesis by liver slices in vitro was estimated through the incorporation of L-alanine U-14C into medium glucose. Tissue preparation, incubation technique, and isolation of glucose-14C from the incubation medium were exactly as previously described (2). Radioactivity measurements were made in a liquid scintillation counter (Beckman, LS-150). The 14C in glycerol was not measured, since preliminary experiments showed that the 14C incorporated into glycerol, isolated from the tissue as described by Abraham and Hassid (1), was less than 5% of that incorporated into medium glucose.

Subcellular Fractionation of Liver and Enzyme Determinations

Determination of PEP carboxykinase activity. The liver was homogenized in ice-cold 0.25 m sucrose (1:9 w/v) with a Teflon pestle homogenizer. After separating an aliquot for enzyme measurement, the homogenate was centrifuged for 10 min at 600 X g, and the supernatant fluid obtained was decanted and recentrifuged at 10,000 X g for 12 min. The sediment of this second centrifugation was washed twice and resuspended to the original volume in 0.25 m sucrose to give the mitochondrial fraction. The cytosol was
obtained by centrifuging the supernatant of the second centrifugation at 105,000 × g for 1 hr. All steps were carried out at 0–3 C. Whole homogenate, mitochondrial fraction, and cytosol were further diluted to give enzyme concentrations within the linear part of the assay system. No increase in activity was obtained by sonically disrupting either the whole homogenate or the mitochondrial fraction for 2 min at 4 C (Branson Sonifier at full power, 4 periods of 30 sec with intervals of 15 sec). Also ineffective was resuspension of the mitochondria in distilled water followed by repeated freezing and thawing. Electron micro- scope examinations of the mitochondrial pellets obtained by the above procedure showed them to consist mostly of mitochondria, with some contamination by lysosomes and portions of endoplasmatic reticulum.

PEP carboxykinase activity was determined through the H14CO3−-oxaloacetate exchange reaction. The assay system was prepared as described by Utter and Kurahashi (22), except that 10 μmoles of MgCl2 were added to 0.5 ml of the reaction mixture. The reactions were stopped by the addition of 5 % trichloroacetic acid. After centrifugation, the solution was gassed for 2 min with CO2, and aliquots were immediately counted in the liquid scintillation spectrometer in Bray scintillator. Blanks in which ITP was omitted were routinely used, and the values obtained were subtracted from all assay measurements.

Determination of Glucose-6-Phosphatase and Fructose-1,6-Diphosphatase Activities

The liver was homogenized in 3 volumes of cold 0.25 M sucrose, and the homogenate was centrifuged for 10 min at 10,000 × g. Portions of the supernatant were assayed for glucos-6-phosphatas and fructose-1,6-diphosphatase activities as previously indicated (2).

Other Chemical Analyses

Liver and pectoral muscle glycogen contents were measured as described by Carrol et al. (3). Glucose was determined by the method of King and Garner (9) in blood collected from the wing vein or by heart puncture. Plasma free fatty acids (FFA) were measured after the manner described by Dole and Meinertz (4).

RESULTS

Table 1 summarizes the influence of fasting on the blood sugar and plasma FFA levels, liver, and muscle glycogen content, as well as on liver and body weight of the black vulture. Blood sugar concentration in the black vulture, as in other birds, is greater than in mammals. Fasting for 72 hr did not affect blood sugar and plasma FFA levels significantly. Liver and muscle glycogen contents were reduced at this time to about 50 and 40%, respectively, of the values for fed animals. After 3 days without food there was a loss of liver and body weight of 20 and 10%, respectively.

The rate of gluconeogenesis by slices prepared from liver of fed black vultures was next compared to that of liver slices from a granivorous bird of similar body size, the domestic chicken. The results (Fig. 1) show that the rate of glucose synthesis from alanine-14C, in vitro, by black vulture liver, is more than twice that of chicken liver. The rate of gluconeogenesis per whole liver, estimated from values obtained in slices incubated for 3 hr, was 256 μmoles/hr for black vulture and 96 for chicken.

The results of the enzyme measurements are given in Table 2. The three enzymes studied, glucose-6-phosphatase, fructose-1,6-diphosphatase, and PEP carboxykinase, were more active in the liver from fed black vulture than in chicken liver. The difference was less marked for fructose-1,6-diphosphatase, when expressed per milligram of liver protein, the activity of this enzyme was only slightly lower in the chicken than in the black vulture. Glucose-6-phosphatase was about 2 times more active in the carnivorous bird. PEP carboxykinase activity was extremely high in black vulture livers, its levels being in average 4 times those of chicken livers. In both species, about 70–80% of the PEP carboxykinase activity of whole-liver homogenate was recovered in the cytosol and mitochondrial fraction; the enzyme was almost equally divided between the two

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\text{TABLE 1. Influence of fasting on blood sugar and plasma FFA levels, liver and muscle glycogen content, and liver and body weight of the black vulture}
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<table>
<thead>
<tr>
<th>Hours of Fasting</th>
<th>Blood Sugar, mg/100 ml</th>
<th>Plasma FFA, μmoles/ml</th>
<th>Liver Glycogen, % Wet Wt</th>
<th>Muscle Glycogen, % Wet Wt</th>
<th>Liver Weight, g</th>
<th>Body Weight, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>167 ± 10 (11)</td>
<td>0.14 ± 0.05 (6)</td>
<td>2.19 ± 0.40 (8)</td>
<td>0.69 ± 0.08 (8)</td>
<td>39.6 ± 2.5 (9)</td>
<td>1.70 ± 0.12 (6)</td>
</tr>
<tr>
<td>24</td>
<td>163 ± 8 (11)</td>
<td>0.46 ± 0.05 (6)</td>
<td>0.43 ± 0.06 (6)</td>
<td>0.28 ± 0.05 (8)</td>
<td>31.6 ± 1.9 (8)</td>
<td>1.61 ± 0.16 (6)</td>
</tr>
<tr>
<td>48</td>
<td>172 ± 9 (11)</td>
<td>0.39 ± 0.04 (4)</td>
<td>1.02 ± 0.24 (8)*</td>
<td>0.28 ± 0.05 (8)*</td>
<td>31.6 ± 1.9 (8)*</td>
<td>1.61 ± 0.16 (6)*</td>
</tr>
<tr>
<td>72</td>
<td>176 ± 9 (12)</td>
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Values are means ± se with number of animals in parentheses. * Significantly different from 0-hr values.
GLUCONEOGENESIS IN A CARNIVOROUS BIRD

Compartments, a little more activity being found in the cytosol. Since the mitochondrial pellet contained other subcellular fragments, it is possible that the actual specific activity of PEP carboxykinase in the mitochondria is higher than that found. It is also possible that part of the activity found in the cytosol comes from enzyme that leaked from mitochondria ruptured during the preparation of the liver cell fraction. However, similar levels of activity were found in cytosol prepared from livers which were homogenized with only one gentle passage of the Teflon pestle.

DISCUSSION

The present studies show that the black vulture, a carnivorous bird, has potentially a high capacity for glucose synthesis. Consequently, the blood sugar levels of these animals are markedly resistant to prolonged periods of fasting, no changes being observed in the blood glucose concentration of the black vulture after a 3-day fast. A similar finding was reported by Nelson et al. (17) in another carnivorous bird, the horned owl, which showed no alteration of the blood sugar levels even when fasted for 7 days. The only granivorous bird that has been studied in detail in this respect is the domestic chicken. In this species fasting of much shorter duration induces a decline of the blood sugar levels (7, 8, 12, 19). It is of interest to note that the increased mobilization of FFA which usually ensues following food deprivation does not occur in the black vulture, whose species has hitherto studied (13). Values for fed rats in this laboratory are 7-10 μmoles/g hr and the black vulture (6.7 μmoles/g hr) are comparatively high. In the rat, utilizing the same technique, values around 2.0 μmoles/g hr were obtained in this laboratory with liver slices from fed animals. It is well established for the rat that liver slices have a relatively low efficiency for gluconeogenesis, higher rates being obtained in the isolated perfused liver (20). This is probably also valid for the avian liver, and therefore the actual rates of glucose synthesis in both the black vulture and the chicken may be higher than those here obtained.

The high gluconeogenic capacity of black vulture liver is associated with higher activities of glucose-6-phosphatase, fructose-1,6-diphosphatase, and PEP carboxykinase, enzymes that are generally accepted as intervening in key steps of the gluconeogenetic pathway by circumventing essentially irreversible glycolytic enzyme reactions (10). The differences were most marked in the case of PEP carboxykinase, which was 3-4 times more active in black vulture than in chicken liver. The levels of liver PEP carboxykinase found in the carnivorous bird are by far the highest of all species hitherto studied (13). Values for fed rats in this laboratory are 7-10 μmoles/min per g liver, almost all of which is localized in the cytosol. The intracellular distribution of PEP carboxykinase is of particular interest, since it has been observed that the location of this enzyme in the liver cell appears to vary from species to species; in some species most (more than 90%) PEP carboxykinase activity is localized in the cytosol, whereas in others a much greater percentage (35-100%) of the total enzyme activity is localized in the mitochondria (10). The present results show that in the black vulture liver, and also in chicken liver,
PEP carboxykinase activity is divided almost equally between cytosol and mitochondria. This distribution is different from that found in another avian species, the pigeon, in which most of the liver PEP carboxykinase activity is localized in the mitochondria (6, 21). The pigeon seems to be the only species so far investigated in which it is possible to obtain net glucose synthesis from lactate, utilizing liver homogenates from black vultures in this laboratory have been unsuccessful (E. Roselino, unpublished observations).

There can be little doubt that the greater effectiveness of gluconeogenesis in the black vulture, as compared to that in the chicken, is directly related to the high-protein and low-carbohydrate content of the food. It certainly constitutes a highly favorable metabolic adjustment to the dietary habits of these animals. In this respect, it is very illustrative to compare the results of the experiments here reported with the recent studies of Eisenstein and Strack (5), on the effect of a high-protein, carbohydrate-free diet on rat liver gluconeogenesis. Among other findings, they observed that rats fed the high-protein diet presented, in comparison to normally fed animals: a) higher levels of blood sugar after 24 and 48 hr without food; b) reduced mobilization of liver glycogen during starvation; c) increased synthesis of glucose from alanine-14C by the liver, and d) higher levels of liver PEP carboxykinase activity. In the present work, all these observations were found to be also true when a comparison is made between a carnivorous and a granivorous bird, utilizing the same metabolic parameters.

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REFERENCES