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LESIONI NUCLEARI IN EPATOCITI DI PRIMATE NON UMANO (CYNOMOLGUS MONKEY) CON DIABETE SPONTANEO

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Riassunto
Una femmina adulta (9 anni) di Cynomologus monkey, non apparteneva a studi tossicologici, presentava all’esame anamnestico una evidente perdita di peso negli ultimi tre mesi. L’appetito dell’animale restava buono e non vi erano altre segnalazioni sintomatologiche. All’esame chimico clinico era presente una evidente iperglicemia (710 mg/dL; range di laboratorio:45-88 mg/dL) e un marcato aumento dei trigliceridi (941 mg/dL; range di laboratorio 28-79 mg/dL). Altre variazioni biochimiche furono osservate nei livelli di colesterolo totale, enzimi epatici ed urea. A livello ematologico era presente una linfopenia significativa. A causa delle precarie condizioni l’animale fu sacrificato. All’esame necroscopico fu osservata una scomparsa delle riserve di grasso corporeo, la presenza di aree scure a livello polmonare con presenza di materiale purulento all’interno dei grossi bronchi. Istologicamente, lesioni patologiche furono evidenziate in diversi organi e descritte come amiloidosi delle isole endocrine del pancreas, broncopolmonite e vacuolizzazione delle cellule renali tubulari. Nel fegato, i nuclei delle cellule epatiche corrispondenti alla zona 1 erano dilatati e contenenti un evidente vacuolo vuoto. Tali nuclei erano simili a quelli descritti in casi di diabete umano o di obesità e descritti come “glycogen nuclei”. La diagnosi primaria proposta e’ un diabete mellito spontaneo con amiloidosi delle isole endocrine pancreatiche.

Parole chiave: scimmia, diabete, fegato

Summary
Hepatic glycogen nuclei in a Cynomolgus monkey with spontaneous diabetes mellitus. A 9-year old female Cynomolgus monkey not currently on study presented with a history of weight loss over the previous 3 months. The animal’s appetite remained good. The most significant clinical chemistry finding was a marked hyperglycemia and hypertriglyceridemia. Because of continuing weight loss the animal was euthanased.

At necropsy severe wasting was evident with an almost complete absence of body fat. Unilateral darkening of the lungs was observed with purulent material in the major airways. Other thoracic and abdominal organs appeared normal. Histopathology revealed changes in a number of organs: severe pancreatic insular amyloidosis, bronchopneumonia and renal tubular vacuolation. In addition, in the liver, nuclei in zone 1 were distended by single, large, discrete, generally empty vacuoles. These were similar to the so-called “glycogen nuclei” described in human cases of diabetes and/or obesity.

A primary diagnosis of spontaneous diabetes mellitus due to insular amyloidosis was made.

Key words: monkey, diabetes, liver, intranuclear inclusions.

Introduction
Amyloidosis is a recognised group of conditions in non-human primates, including macaques. Both focal and multifocal deposition of amyloid proteins can occur. However, whilst pancreatic islet amyloidosis is common in humans (and cats) with diabetes mellitus, it has not been frequently described in non-human primates. In the case we report here amyloid was demonstrable only in the pancreatic islets and was associated with persistent hyperglycemia over many months. A number of intranuclear and cytoplasmic inclusions have been described in hepatocytes of several species, including humans. They can be associated with a number of spontaneous, experimental or iatrogenic conditions, though often their origin remains obscure. In the present case pale hepatocellular intranuclear inclusions were compared to those occasionally described in humans with a number of conditions, including
diabetes mellitus and termed “glycogen nuclei”. The possibility of these inclusions being an artefact are also considered.

**Materials and methods**
The monkey was a 9-year old female Cynomolgus originating from Charles River Primate Laboratory, Mauritius and had been in our small colony for a number of years. It had previously been used in a toxicokinetic study, but the pharmaceutical it had received was not considered to have any influence on the subsequent development of pancreatic amyloidosis. It was singly housed in a stainless steel cage, in a room containing other female Cynomolgus monkeys and fed a regular laboratory chow, supplemented with fruit or vegetables. Drinking water was from the municipal mains supply and given ad-lib from water bottles. Environmental, housing and experimental procedures were in compliance with EEC and Italian Guidelines for Laboratory Animal Welfare.

Clinical chemistry and hematology analysis was performed in-house using a Hitachi 917 and a Bayer Advia 120 analyser respectively.

A complete necropsy was performed; tissues selected for histopathological examination were fixed in 10% normal buffered formalin. Tissues were processed into wax blocks and sectioned prior to staining with either Hematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS) or Congo Red. For H&E and PAS staining sections were cut at a thickness of 4µm; for Congo Red a thickness of 10µm was used. Tissues examined were heart, aorta, lungs, liver, pancreas, kidneys, urinary bladder, spleen, stomach, duodenum, uterus and ovaries.

**Results**
In January 2004 the animal presented with a history of weight loss over the previous 3 months. The animal’s appetite remained good and no other symptoms were reported. Apart from obvious wasting, there were no other signs evident upon clinical examination. A blood sample was taken for clinical pathology analysis (see Table 1).

The most significant clinical chemistry finding was a marked hyperglycemia (710 mg/dL; laboratory range: 28 – 79 mg/dL). Other, less dramatic, changes were seen in plasma concentrations of total cholesterol, liver enzymes and urea. In addition, a significant lymphopenia was present (0.55 x 10³/µL; laboratory range 2.5 – 7.8 x 10³/µL). Retrospective examination of previous routine clinical chemistry analysis performed over the previous 12 months revealed a persistent and increasing hyperglycemia (Table 1). A clinical diagnosis of diabetes mellitus was made. Because of these findings and continuing weight loss the animal was euthanased.

At necropsy severe wasting was evident with an almost complete absence of body fat. Unilateral darkening of the lungs was observed with purulent material in the major airways. Other thoracic and abdominal organs appeared normal. Histological examination showed severe changes in pancreatic islets, with marked reduction in cellularity and replacement by amorphous, eosinophilic material (fig. 1). This material was moderately positive with Congo Red and showed apple-green birefringence under polarised light (fig. 2). Over 90% of islets were affected. A diagnosis of severe pancreatic insular amyloidosis was made.

In the liver, hepatocellular nuclei in zone 1 were distended by single, large, discrete, generally empty vacuoles (figs. 3 and 4). PAS staining showed occasional PAS positive granules within the vacuoles (Fig. 5). Other findings considered incidental were acute bronchopneumonia and renal tubular vacuolation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal range</th>
<th>December 2002</th>
<th>June 2003</th>
<th>January 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>45 – 88</td>
<td>154</td>
<td>345</td>
<td>710</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>28 – 79</td>
<td>166</td>
<td>140</td>
<td>941</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>90 – 147</td>
<td>121</td>
<td>296</td>
<td>176</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>29 – 87</td>
<td>71</td>
<td>54</td>
<td>267</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>26 – 97</td>
<td>40</td>
<td>77</td>
<td>144</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td>917 – 1461</td>
<td>800</td>
<td>202</td>
<td>1991</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>3.8 - 4.9</td>
<td>3.9</td>
<td>3.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.8 - 3.9</td>
<td>3.5</td>
<td>4.3</td>
<td>4.0</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>25 - 54</td>
<td>49</td>
<td>36</td>
<td>147</td>
</tr>
</tbody>
</table>

Table 1: Significant clinical chemistry parameters (December 2002 to January 2004).
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Figure 1. Pancreatic Islet (H&E) showing replacement with amorphous, eosinophilic material.

Figure 2. Pancreatic Islet (Congo Red) showing birefringence under polarised light.

Figure 3. Liver (H&E) showing periportal nuclear vacuolation.

Figure 4. Higher power of figure 3, showing predominantly empty nuclear vacuoles.

Figure 5. PAS stain showing occasional PAS positive intra-nuclear material.

Discussion

A primary diagnosis of spontaneous diabetes mellitus due to insular amyloidosis was made. Clinical chemistry evidence shows that this had likely been developing over the previous year before becoming clinically apparent. The severity of the islet cell changes is consistent with this timescale. Amyloidosis can be secondary to an inflammatory condition. Whilst inflammation was present in this animal, in the form of bronchopneumonia, it was considered too acute to have been a likely initiating factor. In addition, amyloidosis was detected only in the pancreas, whereas a multifocal distribution would be more likely if it was secondary to an inflammatory lesion.
Conclusion
We conclude that this is a case of spontaneous diabetes mellitus secondary to idiopathic pancreatic islet amyloidosis. The changes observed in hepatocytes were considered to be “glycogen nuclei” similar to those described in human cases of diabetes.

References