ROGER DOMINGO OLLÉ

THE GLYCOGEN BODY IN NEONATE BIRDS OF THE ORDER PSITTACIFORMES AND ITS ROLE IN NEONATE MORTALITY

INAUGURAL-DISSERTATION zur Erlangung des Grades eines Dr. med. vet.
beim Fachbereich Veterinärmedizin der Justus-Liebig-Universität Gießen

édition scientifique
VVB LAUFERSWEILER VERLAG

1. Auflage 2006

1st Edition 2006

Printed in Germany

VVB LAUFERSWEILER VERLAG
édition scientifique

STAUFENBERGRING 15, D-35396 GIESSEN
Tel: 0641-5599888 Fax: 0641-5599890
email: redaktion@doktorverlag.de

www.doktorverlag.de

Aus der Klinik für Vögel, Reptilien, Amphibien und Fische
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eingereicht von

Roger Domingo Ollé
Tierarzt aus Barcelona

Gießen 2006

Mit Genehmigung des Fachbereichs Veterinärmedizin
der Justus-Liebig-Universität Gießen

Dekan:	Prof. Dr. M. Reinacher
Gutachter:	Prof. Dr. E. F. Kaleta
	Prof. Dr. K. Frese
Tag der Disputation:	14.11.2006

To my family and friends, which have been there whenever it was.

CONTENT

ABBREVIATIONS:

AcCoA carboxylase: Acetyl–coenzymA carboxylase

ACTH: Adrenocorticotrop hormone

APV: Avian Polyomavirus

ATP: Adenosine–triphosphate

BS: Baby station

CNS: Central nervous system

DIS: Dead–in–shell

EM: Electron microscopy

FL: Fatty liver

FLKS: Fatty Liver Kidney Syndrome

GB: Glycogen body

GI: Gastrointestinal

GLUT1: Glucose transporter-1

HE stain: Haematoxylin – Eosin stain

LED: Late embryonic death

NADPH: Reduced form of nicotinamide–adenine–dinucelotide phosphate

PAS stain: Periodic acid – Schiff reaction stain

PC: Pyruvate–carboxylase

PCB: Polychlorinated biphenyls

PCR: Polymerase – chain reaction

PEPCK: Phosphoenolpyruvate–carboxyquinase

SER: Smooth endoplasmatic reticulum

TG: Thyroid glands

TH: Thyroid hormones

TSH: Thyroid stimulating hormone

T_3: Triiodothyronine

T_4: Thyroxine

YS: Yolk sac

1 INTRODUCTION

Many psittacine species are bred and hand-reared successfully in captivity, including some of the most endangered birds. Techniques and methods have been improved since the beginning of aviculture, and the mortality rate of the nestlings has decreased.

Histopathological examination of dead chicks has played an important role as a tool for the diagnosis of problems and diseases. The study of dead birds in a collection is highly important for the diagnosis and prophylaxis of diseases and management problems. The Loro Parque Fundación, the largest parrot collection in the world, successfully breeds many endangered avian species every year (e. g. *Primolius couloni*, *Ara glaucogularis*, *Rhynchopsitta pachyrhyncha*, *Cyanopsitta spixii*, *Probosciger aterrimus*, etc.), giving the chance of working with a large biodiversity of high ecological value.

Avian neonates are born with a limited quantity of energy stored in the body, and depend on the environment (precocial species) and / or on their parents (altricial species) to get sufficient feed to grow. Although the content of the yolk sac nourishes the neonates during their first day(s) of life, the energy balance during this period is fragile. If there is an imbalance the chick can easily turn into an “energy deficiency” situation which leads to the death of the nestlings.

The glycogen body (GB) is a specific structure on the lumbosacral spinal cord in birds. Its histological structure has been widely studied by many authors (REVEL *et al.*, 1960; MATULINOIS, 1972; LYSER, 1973; SANSONE and LEBEDA, 1976; DE GENNARO and BENZO, 1987), and several hypotheses have been suggested regarding its true function without definite results. A role of energy storage for the spinal cord has been ascribed due to its high content of glycogen derivatives (TERNI, 1924; DOYLE and WATTERSON, 1949; KUNDU and BOSE, 1974).

During the 2002 breeding season in the Loro Parque, from the 1st of January to the 15th of July, 99 dead chicks were studied using macroscopic necropsy and histopathology, and the complex diagnosis of “energy deficiency” was found in many cases. At the histopathological

examination, the GB of 39 chicks was accidentally cut. Surprisingly, no glycogen derivatives were demonstrable in any of them. It is suspected that the absence of glycogen derivatives in the GB could play a role in the cause of death. Solving of the problem might reduce mortality thus enhancing the biological and conservational value of the collection.

A systematic study of all embryos that died during the last days of incubation, and dead chicks up to one month began, evaluating the clinical signs, treatment, postmortem findings and histopathological results to determine the causes of death of the birds. The GB anatomy, histology and pathology were studied, making this research the first and most detailed study on the GB in psittaciformes. The status of the GB, and other structures related with energy metabolism were assessed together because the purpose of the investigation was to clarify the role of the GB in the diagnosis “energy deficiency”.

2 LITERATURE

2. 1 HISTORY

The glycogen body (GB) is a gelatinous glial structure lying in the *fossa rhomboidea spinalis*, in the lumbosacral spinal cord of birds (BREAZILE and KUENZEL, 1993). It was first described in adult birds by EMMERT in 1811, a German researcher, and by NICOLAI in embryos one year later. Since its discovery, the structure has received many different names such as lymph sac, lumbar swelling, fluid body, glial body, sciatic body, gelatinous body (WATTERSON, 1949) and *corpus gelatinosum*, a name which is still commonly used today (BREAZILE and KUENZEL, 1993).

In their studies, different scientists report on the accumulation of large amounts of an unknown material in the cells of the GB (MEYER, 1884; GAGE, 1917) and speculate over the possible nature of the cells forming the structure (DUVAL, 1877). In 1924 TERNI demonstrated, using Best's carmine stain, that the material filling the cells is glycogen. Twenty - five years later, WATTERSON (1949) confirmed TERNI's findings and introduced the term "Glycogen Body". In 1949 DOYLE and WATTERSON further validated TERNI's observations, by applying histochemical and biochemical methods in the domestic chicken.

IMHOF (1905) described the presence of the GB in more than 30 different avian species, aquatic and terrestrial. It has also been found in some dinosaurs (Stegosaurs) as an enlargement of the spinal cord in the hip vertebrae. MARSH[1] and others suggest that the lumbosacral sinus in these species housed a second brain (to control the back legs and tail). A more credible hypothesis has been proposed by GRIFFIN[1] who relates the lumbar enlargement of the dinosaurs with the one in the birds. ZAMORA (1978) reports stored glycogen in specialised glial

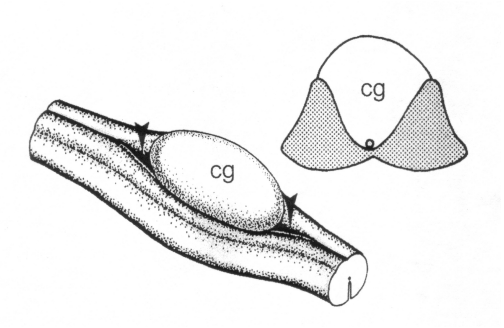
[1] 2003 Internet information.

cells along the spinal cord of an amphibian species, the Ribbed Newt (*Pleurodeles waltlii*), with many similarities to the avian lumbosacral GB and the avian brachial GB (see page 16).

2. 2 ANATOMY OF THE GLYCOGEN BODY

2. 2. 1 Gross anatomy

The GB in chickens is in a dorso-ventral view an oval-shaped mass between the dorsal columns of the spinal cord at the level of the roots of the sciatic nerves (LYSER, 1973)(See Picture 1). It is confined to a small area between the third lumbar and first sacral vertebrae in the *fossa rhomboidea spinalis*. On transverse sections of the spinal cord it has an inverted triangular shape, which reduces the connection of the nervous tissue to a small ventral portion (WATTERSON, 1949)(See Picture 1). The *canalis centralis* passes through the ventral apex of the GB in the longitudinal axis, and the ependyma is encircled without interposition of a basement membrane (WELSCH and WÄCHTLER, 1969; MÖLLER and KUMMER, 2003).



Picture 1: Dorso-ventral view and transveral section of the chicken chick glycogen body (*cg* corpus gelatinosum). (MÖLLER and KUMMER, 2003 ©).

The meningeal localisation of the GB has been widely discussed. HANSEN – PRUS (1923) and KAPPERS (1924) state that the GB lies between the leptomeninges, while DUVAL (1877), KÖLLIKER (1902) and WATTERSON (1949) assume that its position is wholly subpial. Contrary to all these statments, DICKSON and MILLEN (1957) finally describe two portions of the GB, using adequate stains for the meninges. These are the dorsal, intrapial portion, which is enclosed by a pial septum, and the ventral, subpial portion, which is situated under the pial septum and is in direct contact with the spinal cord tissue. They have also found that the structure is completely surrounded by the pial septum near the cranial and caudal poles of the GB.

2. 2. 2 Histology

Under optical microscopy the GB cells present themselves as large, irregular polygons with rough edges. Most of the volume is taken up by a moderately dense to what appears to be almost empty area (LYSER, 1973). When stained with Best's carmine (TERNI, 1924; WATTERSON, 1949) or periodic acid-Schiff stain (PAS) (DE GENNARO, 1959), a bright red coloration in the "empty areas" reveals the presence of glycogen derivatives filling the space. The nucleus and the perinuclear cytoplasm are usually found at one edge of the cell, displaced by the large amounts of stored glycogen derivatives. The boundaries between the cells forming the GB are evident at optical microscopy (LYSER, 1973).

The vascularization of the GB is rich and the glial cells are in direct contact with the basal lamina of the blood vessels (LYSER, 1973). The capillaries have a continuous endothelial wall, and the astroglia of the GB forms a loose barrier with a wide pericapillary space (AZCOITIA *et al.*, 1985). MÖLLER and KUMMER (2003) have demonstrated the existence of a blood–brain barrier by using intravascular injection of tracers and the immunocytochemical detection of functional and structural markers.

PAUL (1971, 1973), using fluorescent histochemical techniques, demonstrated the presence of an aminergeric net coming from the autonomous nervous system that innervates the GB.

2. 2. 3 Ultrastructure

Various ultrastructural studies provide good descriptions on the cell structure of the GB (REVEL *et al.*, 1960; LYSER, 1973) and its development (MATULINOIS, 1972). In 1963, REVEL studied the structure of the glycogen in different tissues (including the GB) with electron microscopy (EM) and identified two kinds of glycogen particles, called β (spherical single particles of 150 – 400 Å) and α (complex units of packed mass particles which can reach up to 0,1 μ in diameter), with a wide range of sizes inbetween. He reports that the glycogen in the GB is presented in single particles. In agreement with this, MATULINOIS (1972) describes the single elements similar to β-particles in later ultrastructural studies. The bigger particles have always been seen in lesser numbers than the small ones, and while some authors call them α-particles (MATULINOIS, 1972) others do not (LYSER, 1973). The stored glycogen derivatives in the GB are intracellular and free of packing membranes (LYSER, 1973).

LYSER (1973), in her detailed study in chicks, describes the organisation of the cell structures: The nucleus is rather dense, as is the cytoplasm both perinuclearly and in a slim rim along the cell membrane. The nucleus is rounded, elongated and somewhat irregular in shape. The high number of ribosomes gives the cytoplasm its appearance of high density. Many of the cellular organelles can be found perinuclearly, such as the granular endoplasmic reticulum, the Golgi complex, mitochondria, multivesicular bodies, and small groups of cytoplasmic filaments. The glycogen containing area is free of organelles, except for some rare filaments and some ribosomes.

The ependymal cells of the central canal are the same in the GB as in the rest of the spinal cord (LYSER, 1973). The presence of rounded profiles resembling glycogen particles in the central canal has been reported by some investigators (LYSER, 1973; AZCOITIA *et al.*, 1985). WELSCH and WÄCHTLER (1969) in their ultrastructural study of the GB in pigeons describe GB cells crossing the ependyma to reach the central canal, while LYSER (1973) did not see that in chicks. Bulbous protrusions and a high content of glycogen in the ependymal cells of the GB are reported by AZCOITIA *et al.* (1985) in hatched chicks and embryos. The same author *et al.*, also describe these profiles in the central canal, in the perivascular space and even in the lumen of

capillaries including those in the nearby nervous tissue. Low concentrations of glycogen were seen in the perivascular space with the endothelium showing secretory specialisations (like great vacuoles and evaginations), and high concentrations of glycogen particles inside the vessels (AZCOITIA *et al.*, 1985).

An electron microscopic study of the innervation of the GB showed synapses on the surface of GB cells (WELSCH and WÄCHTLER, 1969). Later, PAUL (1971) using fluorescent and silver impregnation stains reports that the astrocytes are innervated by non-myelinated axons. Also, large multipolar ganglion cells have been seen dispersed in the outermost border of the GB as in the inner half of the adjacent nuclear area. Dendritic processes have been seen penetrating into the centre (SANSONE and LEBEDA, 1976). MATULINOIS (1972) in his ultrastructural study on the developing GB has never observed nerve processes among the cells, which could be due to inappropriate stains. Studies on the developing GB in Japanese Quail (*Coturnix japonica*) (DE GENNARO and BENZO, 1987) revealed abundant, extremely fine nerve fibres from the grey matter demonstrated by Bodian's silver staining method. With HE stains these fibres escape detection.

Boundaries between GB cells and the neural tissue, the nerve bundles and the ependyma have not been reported (MATULINOIS, 1972; LYSER, 1973). MATULINOIS (1972) in his ultrastructural study of the developing GB in chickens has not seen junctions between the GB cells, either. Local temporary enlargements in the intercellular space of the GB cells have been described (DE GENNARO and BENZO, 1978; MÖLLER and KUMMER, 2003).

The GB seems to be an integral part of the central nervous system, and to be composed by a special form of astrocytes (LYSER, 1973; LEE *et al.*, 2001), like some other scientists have suggested before (KÖLLIKER, 1902; IMHOF, 1905). Even so, there are some important differences between astrocytes and GB cells (apart from the storage of glycogen), such as the high quantity of ribosomes and granular endoplasmic reticulum, which is less frequent in normal astrocytes. The high density of the nucleus and cytoplasm is also unusual in normal astrocytes.

2. 2. 4 Possibly related structures

The accessory lobes (also known as the Lachi lobes) of the spinal cord consist of segmentally arranged protrusions which extend bilaterally from the lumbosacral cord (LACHI, 1899). The structure is similar to the GB except for a high number of large myelinated nervous cells (LACHI, 1902). Following his discovery of the glycogen stores in GB cells, TERNI (1924, 1926) mentions a possible relationship with the Lachi lobes because of their similar composition on glycogen–rich cells. DE GENNARO, BENZO *et al.* (1975, 1978, 1981), in their consecutive studies on the GB, also establish ultrastructural and enzymatic characterizations of the Lachi lobes which suggests a close relationship between both structures. The enzymes found in the Lachi lobes have been shown to be the same as in the GB demonstrating the tissue capability for metabolising glucose by the pentose phosphate cycle, and may have a possible role in the myelin synthesis (BENZO and DE GENNARO, 1981).

SANSONE and LEBEDA (1976) describe in the domestic chicken a group of cells around the central canal in the cervical region with a considerable amount of glycogen stored. They regard those cells as specialised astroglia similar to the GB cells, but with less content in glycogen. The structure has been called brachial GB and seems to correspond to the ventral portion of the lumbosacral GB. Histochemically, the brachial GB reacted similarly to the lumbosacral GB. The authors also suggest that this structure could be found along the whole spinal cord (like in the Newt described by ZAMORA in 1978), supporting the hypothesis of the GB as a source of glucose for the cerebrospinal fluid (see page 22). In further studies, SANSONE (1977, 1980) confirms the – not very pronounced – craniocaudal extent of the brachial GB along the spinal cord up to the oculomotor level, surrounding the central canal and consisting of intermediate astroglial type cells.

2. 3 ONTOGENIC DEVELOPMENT OF THE GLYCOGEN BODY

The development of the GB in the avian embryos was firstly studied by NICOLAI in 1812, and subsequently by many others (WATTERSON, 1949; WATTERSON and SPIROFF, 1949; WATTERSON, 1952, 1954; WATTERSON, VENEZIANO and BROWN, 1958; DE GENNARO,

1959; MATULINOIS, 1972; DE GENNARO and BENZO, 1987, 1991). The earliest indication of formation of the GB found in the nerve cord of the chicken embryo is at 7.5 – 7.75 days of incubation (MATULINOIS, 1972), with some slightly stained cells being visible using Best's carmine or PAS stains and diastase as a control. Also in the embryos of the Japanese Quails (*Coturnix japonica*) the GB appears first at 7 – 8 days of incubation, even though the incubation period is shorter than in chickens. DE GENNARO and BENZO (1987) suggest that this similarity could be due to the penetration of blood vessels in the roof plate, which could mediate the development. The GB originates from bilateral clusters of cells in the roof plate (WATTERSON, 1952, 1954) as a wedge–shaped mass in the dorsal aspect of the spinal cord. Recent studies indicate that the PAS-positive cells arise from the neuroepithelium that comprises the ependyma and the roof plate of the avian lumbosacral spinal cord (LOUIS, 1993).

The first ultrastructural study on the developing avian GB was carried out by MATULINOIS (1972) in chicken embryos. The number of GB cells since its appearance increases in number until day 15 of incubation (MATULINOIS, 1972), when the GB starts surrounding the central canal (DICKSON and MILLEN, 1957). At 10 days the primordia are totally fused and at day 11 the GB can be seen without visual aids. From 10 to 14 days of incubation glycogen is stored steadily, and from day 15 to 18 the volume of the cells increases, packing the glycogen more densely. Then the cell cytoplasm divides in three areas: a glycogen–free region with the nucleus and the organelles, a peripheral cytoplasmic area also free of glycogen, and a large region with glycogen particles densely packed (MATULINOIS, 1972). The GB accumulates glycogen, accounting for 60-80% of its dry weight, during the remainder of the embryonic development and after hatching (DOYLE and WATTERSON, 1949). DE GENNARO (1961) reports that the GB cells at day 19 of incubation are primarily filled of glucose and no other carbohydrates. Ribosomes were found attached to, or in close proximity to glycogen deposits (MATULINOIS, 1972), and it is known that they produce the enzymes for the glycogen synthesis (HEUSON–STIENNON and DROCHMANS, 1967).

Also the smooth endoplasmic reticulum (SER) was seen morphologically close to the glycogen particles in periods of deposition and breakdown (DE GENNARO and BENZO, 1991). The Golgi complex is another prominent organelle in the GB cells, but its major development takes place during the later stages of incubation (day 12 – 18). It has always been found at a

certain distance from the glycogen deposits and the relationship with the stored glycogen is unclear. It has been suggested that the Golgi complex produces “C-shaped” multivesicular bodies of an unknown function (MATULINOIS, 1972).

The blood–brain barrier of the GB is completed at 15th day of incubation in chicken embryos as demonstrated by MÖLLER and KUMMER (2003).

2. 4 GLYCOGEN BODY METABOLISM

In an attempt to learn about the function of the GB and its metabolism, many scientists have studied the synthesis and lysis of glycogen (DE GENNARO, 1962; SNEDECOR *et al.*, 1962) and the enzymes of the GB cells (HAZELWOOD, 1965; DE GENNARO, 1974; BENZO *et al.*, 1975; FINK *et al.*, 1975; KUMAR and SINGH, 1982). Experiments with glucose [^{14}C] have been carried out to see if the GB is able to incorporate and release glucose. It has been concluded that the GB incorporates glucose and the glycogen stored can be degraded and utilized by the chick when grafted to the chorioallantoic membrane (DE GENNARO, 1974). Probably, the blood supplied the glucose necessary for the synthesis of glycogen derivatives in the GB cells and it crosses the blood–brain barrier by using the glucose transporter-1 (GLUT1) of the endothelium (MÖLLER and KUMMER, 2003).

HAZELWOOD *et al.* in 1962 was the first to report the presence of glycogen phosphorylase by histochemical methods in the GB. LERVOLD and SZEPSENWOL (1963) suggest that the glycogenolytic activity of the GB is low due to the poor content in glycogenolytic enzymes. BENZO and DE GENNARO (1974) continued the enzymatic studies and describe the occurrence of glycogen synthetase and phosphorylases, which become more active in the presence of their metabolites in the GB. Their conclusions are that the GB can synthesize and degrade glycogen but the turnover (synthesis and lysis) is little or non-existent. In 1975 BENZO *et al.* confirms the presence of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These enzymes were found to have higher activities (2–5 times greater) than in the liver and muscle. Another finding is the lack of glucose-6-phosphatase (the enzyme necessary for the transport of glucose from the inside to the outside through the cell membrane) in the GB. All

these results have produced new hypotheses such as the role of the GB in the myelin synthesis using the direct oxidative pathway (pentose phosphate cycle). An interesting study was carried out by BENZO and DE GENNARO (1981), analysing and comparing the existence of glycogen synthetase, glycogen phosphorylase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and the lack of glucose-6-phosphatase in the GB, liver, muscle and Lachi lobes (see page 16). They have found higher activities in the Lachi lobes and GB of glycogen synthetase and glycogen phosphorylase independent from their metabolites, than in the liver and muscle. The enzymatic activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the Lachi lobes were also found to be greater than in the liver and muscle. The lack of glucose–6–phosphatase has been reported as a common feature of the Lachi lobes, the GB, and muscle. In conclusion, BENZO and DE GENNARO (1981) state that the GB produces more pyruvate and lactate than the liver.

The factors controlling the GB metabolism are still uncertain. Hormonal testing and starvation have been widely investigated. Different studies have been carried out in order to demonstrate the emptying of the GB under starvation conditions without success (SZEPSENWOHL and MICHALSKI, 1951; SMITH and GEIGER, 1961; HOUSKA *et al.*, 1969). Just GRABER *et al.* (1972) and KUNDU and BOSE (1974) report a loss of glycogen in such situations.

Endocrine tests using hormones affecting the carbohydrate metabolism have also produced conflicting results:

- Exogenous epinephrine has no effect on the GB according to SNEDECOR *et al.* (1962), while BUSCHIAZZO *et al.* (1964) report an increase of glycogen content in the GB in a study with ducks, in which the animals lost weight by unknown causes.
- All authors agree that glucagon has no effect on the content of the GB (HAZELWOOD *et al.*, 1962; SNEDECOR *et al.*, 1962).
- WATTERSON *et al.* (1958) studied the effect of hypophysectomized chicks on the GB content and report a decrease of the storage in the GB. HAZELWOOD *et al.* (1962) agrees with these results in his investigation describing an increase in the content of glycogen in the GB, by using external supplementation of avian

hypophyseal extract. Contrary to these authors, THOMMES and JUST (1966) have found no differences in the content on glycogen of the GB comparing control and hypophysectomized chicks.

- It has been shown that insulin has a transient effect on the GB content of glycogen which is suspected to be indirectly mediated by avian adrenocorticotropic hormone (ACTH) (HAZELWOOD *et al.*, 1962). Later investigations by the same scientist showed no effect with either intravenous or intracysternal injections (ANDERSON and HAZELWOOD, 1969).
- HAZELWOOD *et al.* (1962) notes an increase in glycogen content of the GB by external supplementation with mammalian ACTH.

In an attempt to understand the inefficacy of hormones on the GB, different possibilities have been suggested, one example being that the GB is beyond the hormonal sphere of normal metabolic pathways (HAZELWOOD *et al.*, 1962), or that it is a vestigial structure (IMHOF, 1905; JELGERSMA, 1951), or that the polysaccharides in the GB have a different configuration from the carbohydrates of other tissues (SZEPSENWOHL and MICHALSKI, 1951; HAZELWOOD et *al.*, 1962). None of these theories have been proven so far.

The capacity of the GB cells to synthesise and to store glycogen without being under hormonal control has been demonstrated *in vitro* by De GENNARO in 1959.

PAUL (1972, 1973) achieved depletion of glycogen from the GB following subcutaneous administration of some pharmacological agents such as convulsants, strychnine, or charbacol (acetylcholine analogue), and an increase in its quantity, when methamphetamine (noradrenalin agonist) is used.

Recent publications (LEE *et al.*, 2001) show that the glycogenolysis of GB cells *in vitro* is partially affected by α-adrenergic receptors (possibly not by direct effect), but not by β-adrenergic receptors. Some authors suggest a possible neural control of the GB (WELSCH and WÄCHTLER, 1969; PAUL, 1971, 1973).

SZEPSENWOL (1953) reports an increase in size of the GB in chicks fed high-protein diets, and a decrease in low-protein diets. He concludes that the GB cells, compared to other astrocytes, are extensively equipped for the synthesis of proteins. LYSER (1973) deduces from these results that there might be some protein associated with the glycogen stores.

The mechanical manipulation (WATTERSON, 1954) or destruction by cauterization (JENKINS, 1955) of the GB primordia in the embryo was carried out producing only disorganisation or the complete loss of the structure with no effect on the growth of the limbs. Only JENKINS (1955) informs that a decrease in liver glycogen that normally takes place after 12 days of incubation is less pronounced in these chicks, but he based his study solely on the histochemistry of tissue sections. Other investigators (SAUER, 1962; PIERCE and FANGUY, 1971; FERNANDEZ - SORIANO *et al.*, 1981) worked in chickens after surgical removal of the GB, studying possible changes in the behaviour (such as under stress situations) and the effects of hormonal supplementation. However, they did not find differences compared to control animals.

2. 5 HYPOTHESES ON THE FUNCTION

Several hypotheses have been forwarded during all these years of studies on the GB, but there is not yet a clear idea of the real function. When the glycogen in the cells was at first discovered, many authors thought of an energy store for the spinal cord to be used in special situations such as starvation (TERNI, 1924; DOYLE and WATTERSON, 1949; KUNDU and BOSE, 1974), prolonged exercise or migratory flight (WELSCH and WÄCHTLER, 1969). However, in experiments on the effect of starvation on the GB content, this could not be proved.

The mammalian central nervous system (CNS) has been found to use lactate and ketone bodies in starvation and stress situations (OWEN *et al.*, 1967; ROLLESTONE and NEWSHOLM, 1967). The glycogenolytic pathway in the GB produces lactate, pyruvate and ketone bodies, which might be used by the nervous system in stress or starvation situations (BENZO and DE GENNARO, 1981).

Other researchers describe glycogen in neuronal cells to be implicated in synaptic transmission (DRUMOND and BELLWARD, 1970). IBRAHIM (1972) concludes from his studies with hypoxic and ischemic brains that the areas of the CNS with high glycogen are generally vulnerable to metabolic disturbances.

Another theory proposed by SMITH and GEIGER in 1961 (and supported by WELSCH and WÄCHTLER, 1969) is that the GB provides glucose for the cerebrospinal fluid. AZCOITIA *et al.* (1985), in their article "Is the Avian Glycogen Body a Secretory organ?", suggest an apocrine mechanism of the GB for the secretion of glycogen (see page 14 and 15). On the other hand, PAUL (1971) thinks of the cerebrospinal fluid as a source of glucose for the GB.

After the enzymatic characterization of the GB cells, another hypothesis regarding the function of this mysterious structure has emerged, which is the synthesis of myelin. The presence of the necessary enzymes for the pentose phosphate cycle, the most important pathway for the synthesis of the reduced form of nicotinamide–adenine–dinucleotide phosphate (NADPH), has also been demonstrated by BENZO *et al.* (1975). The NADPH is an indispensable cofactor in some synthetic reactions forming long–chain fatty acids and cholesterol (HOLLMAN, 1964) for the myelin formation (BENZO *et al.*, 1975; FINK *et al.*, 1975). BENZO *et al.* (1975), supporting this hypothesis argues that it has been demonstrated that the myelinization of the avian nervous system starts at day 14 of incubation, which is when the glycogen synthesis in the GB sharply increases.

IMHOF (1905) thinks that the GB is just a non-functional remnant of the "sacral brain" from the reptilian ancestors.

Other less documented hypotheses are:

- SANSONE (1977), following his study on the craniocaudal extent of the GB in domestic chickens, suggests a functional involvement with general somatic efferent neurons.

- The cells of the ventral horn of the chicken spinal cord have phosphorylase activity suggesting that the GB could be a possible backup source of energy for the motor neurons involved in the synaptic mechanisms (SANSONE, 1977).
- The presence of argentaffin cells in the GB can also indicate a possible neurosecretory function (FREWEIN, 1992).

2. 6 ENERGETIC METABOLISM OF EMBRYOS AND CHICKS

The composition of the eggs varies from altricial to precocial birds, and from species to species, but generally the concentration of carbohydrates is low. For example, the nutritional value of a chicken egg (average 50g) is: 0.6g carbohydrates, 5g fat, 6.3g proteins and small amounts of vitamins and minerals (COUTSS and WILSON., 1990). The yolk composition varies but is always rich in lipids, and its water content ranges from 40% (precocial species) to 65% (altricial species). The water content of the albumen is more constant (88–90%). Summarising the egg composition (yolk + albumen), altricials usually have more than 80% in water content and an energetic density of less than 5kJ/g (5.35kJ/g on average in psittaciformes), while in the precocial eggs the water content is normally under 75% and energy density is between 6 and 12kJ/g. The variability of the energy density is mainly due to the water content (VLECK and BUCHER, 1998).

Adenosine–triphosphate (ATP) is mainly produced via the Krebs cycle (citric acid cycle) in the mitochondria, and the main source for this cycle to produce energy are carbohydrates. 80% of the glucose in the breeding egg is used during the first six days of incubation (TUSCHY, 1983). The lipids and proteins can also be used for the production of glucose, but firstly they need to be converted by the gluconeogenic pathway.

When the oocyte is fertilized by the spermatozoa, the cells start to replicate, needing energy to grow, and to differentiate into the various cell groups. During the first eight days of life, the chicken embryo gets glucose from the latebra, also called white yolk (CHRISTENSEN, 1997). The embryos feed on the yolk and after having finished the organogenesis they start swallowing albumen and digest it for their growing process. At hatching they start using the still large amount of yolk in the yolk sac by absorbing it via the blood and also by intestinal digestion. Since the

concentration of carbohydrates in the albumen and the yolk is low, the gluconeogenesis is necessary to produce glucose from lipids and proteins. The levels of glucose in the blood of embryos are generated by gluconeogenesis of the yolk lipids and proteins (TUSCHY, 1983), which enter the process as lactate, pyruvate or oxalacetate after their lysis. It has been experimentally shown that the embryonal chick liver is incorporating pyruvate, alanine and glutamate for the gluconeogenesis on days 10, 14 and 17 of incubation (TUSCHY, 1983). In the first steps of the gluconeogenesis, lactate is converted to pyruvate, which is transformed to oxalacetate by the enzyme pyruvate-carboxylase (PC), and finally turned into glucose, which can then enter the citric acid cycle to produce ATP. The activity of pyruvate–carboxylase and phosphoenolpyruvate–carboxyquinase (PEPCK) has been studied in embryonal liver and considerable increases have been seen. The activities of these enzymes in the liver of embryos at day 8 of incubation are higher than in the liver of adult chickens by 46% for PEPCK and 39% for PC, respectively. By day 17 of incubation, the respective enzyme activities rise to 64% and 60% above the values for those in the liver of adult chickens (TUSCHY, 1983). The biotin–dependent PC is a key–enzyme in the first steps of the gluconeogenesis. Also the activities of glucose–6–phosphatase and fructose–1,6–biphosphatase are 50% higher in the embryonal liver than in the adult chickens. After hatching, these values decrease in the liver but the PC activity increases strongly up to day 16 of life, where it levels out until day 30.

The acetyl coenzyme A carboxylase (AcCoA carboxylase) is another biotin–dependant enzyme, and is one of the main enzymes in the citric acid cycle for the production of ATP. AcCoA carboxylase is also implicated in the synthesis of fatty acids (MURPHY, 1992). The other enzymes which depend on biotin are: propionyl coenzyme A carboxylase (necessary for the use of volatile fatty acids produced in those birds with ceca); and charbamoyl phosphate synthetase (for the production of pyrimidine nucleotides) (KOLB, 1992). PC is much more affected by biotin deficiency than AcCoA carboxylase (PEARCE and BALNAVE, 1978).

2. 7 BIOTIN IN BIRDS

Hexahydro–2–oxo–1H–thienol [3,4-d] imidazole–4–pentonic acid, also called biotin or vitamin H, is covalently linked to proteins in most of the avian feeds (KLASSING, 2000). Biotin is also

covalently linked to lysine as biocytin, and requires biotinidase (secreted by the pancreas and mucosal cells) for proteolytic release of free biotin. Only the D isomer of the biotin has vitamin activity and is converted inside the cells to carboxybiotin (KOLB, 1992). Biotin is absorbed in the small intestines, but many sources of protein-bound biotin are resistant to digestion, turning the biotin in one of the least available vitamins. The microflora in the intestines of the chickens synthesizes biotin, but its absorption from the lower jejunum is restricted or impossible. Biotin is especially important as a cofactor for enzymes involved in processes such as gluconeogenesis (PC), energy metabolism (AcCoA carboxylase), lipogenesis (AcCoA carboxylase) and elongation of essential fatty acids (KLASING, 2000).

In the blood, biotin is transported by the biotin–binding protein I, and small amounts of it can be found in the yolk of the egg (KLASING, 2000). The vitamin H is stored in the liver, sustaining the animal for a few weeks if there is no absorption of the molecule (BRYDEN, 1989). In the egg, biotin can be found in the albumen and in the yolk. Biotin–binding protein II is the most frequent storage form of the vitamin in the yolk and its main source for the developing embryo. This protein is synthesized by the liver of the hen and binds biotin with high affinity, but not covalently. In the albumen, biotin is not available because it is bound with high affinity to avidin, a protein with antibacterial properties (KLASING, 2000). Avidin can bind three mols of biotin. Three types of avidin have been described, A, B and C, the last one of which is at a lower percentage in the albumen than the other two. Avidin A and B have the same capacity for binding biotin, the only difference lying in the composition of amino acids. Avidin is secreted by the global cells of the magnum in the reproductive tract of the female. It is also present in the albumen of fish and frogs and it is generally believed that it is of particular importance in reproduction. Biotin–binding protein II in the yolk delivers biotin 100 times quicker than the avidin of the albumen (MEHNER, 1983).

When embryos swallow albumen, the avidin can bind biotin present in the gastrointestinal tract. It has been postulated that day-old chicks may contain high levels of avidin in their yolk sac and this may reduce the available biotin, leading to a reduced hepatic gluconeogenesis (DAVIES, 2000). This has not been confirmed by other authors.

There is a positive relationship between the biotin level in the diet of the hen and the concentration in the egg yolk (WHITEHEAD, 1984). High levels of biotin in the egg provide stores in the chick that buffer a dietary deficiency for at least one week (KLASING, 2000). The liver concentration of biotin in neonates will depend on liver reserves at hatching, the strain or species of the bird, and the diet it is fed (BRYDEN, 1991).

As nutritional sources of biotin, organs such as liver and kidney, the vegetative part of plants, legumes (peanuts and soybeans) and some fruits can be utilised. However, as soon as stored feed becomes rancid, it will loose its biotin content (KLASING, 2000).

Evaluation of biotin requirements for birds is difficult due to the variable availability of the vitamin. Estimated biotin requirements for chickens, quails, turkeys, geese, ducks and pheasants range from 100 to 300 µg/kg food. Requirements for psittaciformes have not been established, but an interesting study on the composition of commercial diets for parrots reports biotin levels ranging from 88 to 1000 µg/kg (BAUCH, 1995).

Factors controlling the microflora, such as antibiotics, can also play a role in the production and absorption of intestinal biotin produced by bacteria. Clinical biotin deficiencies are rare, except when diets are based on grains with low biotin bioavailability or when antagonists (such as avidin) are present in the diet (KLASING, 2000). Deficiencies have been seen more often in young pairs, which have been related to the fact that some of them are egg–eaters. In diets containing raw egg, the availability of the vitamin also sharply decreases following the presence of avidin since its complex with biotin is resistant to digestion (WHITE *et al.*, 1992). Avidin is destroyed by heat, thus availability of biotin can be improved by heating.

Biotin deficiency appears to be closely related to the Fatty Liver Kidney Syndrome (FKLS) in chickens. Both early and late embryonic deaths have also been described with biotin deficiencies together with dwarfism, ataxic chicks, chondrodystrophy and deformities of the beak and skeleton (KLASING, 2000). Furthermore, biotin deficiency in growing chicks inhibits the development of lymphoid tissue (KOLB, 1997).

2. 8 NEONATAL ADAPTATION

In this section some literature, important for the histopathological evaluation, is cited regarding the transformations which take place pre- and post- hatching:

2. 8. 1 Influence of the thyroid gland

The thyroid glands (TG) and their hormones are well known. Especially in embryos, the thyroid hormones (TH) regulate the growth and maturation of many tissues, govern the metabolism and also have an important role in the thermoregulation of neonates. Thyroxin (T_4) is the main hormone produced and stored by the TG. Its activity seems to be low and during the incubation it reaches higher levels than triiodothyronine (T_3). T_4 is transformed to T_3, the most active form, by the 5'-monodeiodinase enzyme in the liver and other tissues peripheral to the TG (DECUYPERE, 1993; McNABB and CHENG, 1985).

TH have been the subject of many studies attempting to find the main differences in the ontogenesis between precocial and altricial birds. The formation of the TG starts on the second day of incubation (STEINKE, 1983). Studies on quails (as precocial species), doves and starlings (as altricial species) made by McNABB and collaborators (1984, 1985, 1988, 1996, 1997) have revealed two different patterns, depending on time and the developmental state of the hatchling. For precocials the authors describe rapid maturation of the TG prior to hatching and a peak of T_3 concentration in the perinatal period. The transformer enzyme seems to be responsible for this peak, rather than any increase in the production of T_3. The maturity of the TG at hatching makes the endothermic response to cooling during the last period of incubation possible, which is characteristic in the precocial species. In altricial birds on the other hand the activity of the TG is lower. They do not present a peak of T_3 at hatching and this is reflected in the absence of the endothermic response. When altricial birds leave the shell, the TG have a low turnover and tend to release the hormones instead of storing them. This causes a rise in T_3 and T_4 serum concentrations during the first 8 days of life, thus increasing the thermoregulatory ability over time. In their studies, the authors report a highly functional TG at day 15, and an increased production and storage of hormones compared to the birth date.

The brain and liver of chicken embryos present receptors for TH by day 7 and 9 of embryogenesis, respectively (BELLABARBA and LEHOUX, 1981; HAIDAR and SARKAR, 1984). The TH activate the synthesis of microtubule-associated proteins necessary for the organisation of the brain in different layers (NUNEZ, 1984), and may encourage the maturation of cells (McNABB, 1995).

The TH also play an important role in the humoral immunity, because physiologic levels of TH are necessary to maintain normal weights of the *cloacal bursa* and thymus (BACHMAN and MASHALY, 1986). Also the withdrawal of the yolk sac into the coelomic cavity and the pulmonary vascular resistance prior to hatching is correlated to the level of thyroid hormones (DECUYPERE, 1993).

The TH exert many other important effects in the growing bird, for example on the development of functions such as the digestion, the absorption and the biochemical processing of feed during the post-hatching period. Other effects are on the development of the skeletal system and on the maturation of the lungs (McNABB *et al.*, 1998).

2. 8. 2 Development of the immune system

In the chicken, cellular precursors of the immune system have a mesenchymal origin. The stem cells migrate from the dorsal mesentery of the abdomen to the yolk sac membranes, and there the haematopoietic tissue responsible for erythropoiesis and granulopoiesis establishes. Later, the haematopoietic tissue also starts building the bone marrow inside the precursors of the bones, and from there, the pre–B–cells and the pre–T–cells colonise the *cloacal bursa* and the thymus, which supply an epithelial network for differentiation into specific lineages. The colonisation of the *cloacal bursa* takes place typically between day 8 and 14 of incubation, although cells which colonise the *cloacal bursa* can be found in the bone marrow until hatching. The cells colonising the *cloacal bursa* undergo a selection according to their responsiveness at antigen presentation: all cells not reacting to presented antigens and all cells heavily reacting to the antigens are killed by apoptosis. This concerns 90 – 95% of the cells. After this selection, the

remaining cells start to proliferate heavily and produce the B–cell pool. Some of these B–cells from the *cloacal bursa* colonize peripheral lymphoid tissues and will be the source of antibodies. The importance of the *cloacal bursa* lies in its role in the creation, diversification and expansion of the antibody range that the bird will use during the rest of its life (TIZARD, 2002). In cockatiels (*Nymphicus hollandicus*) the growth of the *cloacal bursa* has been recorded up to the 21st day, then the volume decreases and fluctuates until its involution. The involution of the *cloacal bursa* takes place when the bird becomes sexually active (ITCHON and LOWENSTINE, 1997).

The thymus is colonised by T–cells in successive waves at days 7, 12 and 18 of incubation. These waves last for 36 hours after which they are interrupted and followed by non–receptive periods (APANIUS, 1998). After selection the T–lymphocytes migrate as mature lymphocytes (T–helper cells and cytotoxic T–cells) to secondary lymphoid tissues (pers. comm., Prof. Gerlach). The thymus growth of the cockatiels (*Nymphicus hollandicus*) is fluctuating after hatching (ITCHON and LOWENSTEIN, 1997).

The spleen is an organ that develops quite early in embryonic life, appearing between day 4 and 5 of incubation in chickens according to HAMILTON (1952). At day 8, the spleen has the same level of development as the bone marrow has at day 12 (LUCAS and JAMROZ, 1961). At the beginning of the last century, DANTSCHAKOFF (1908) stated that the blood forming function of the bone marrow starts around day 14 of incubation. The lymphocytes are the dominant cells of the chicken spleen at the second day after hatching (LUCAS and JAMROZ, 1961).

2. 8. 3 Development of the haematopoietic system

During the first days of incubation the yolk sac membranes are the main site of erythropoiesis. The need for red blood cells is rising while the yolk sac size decreases and the embryo grows. Following this period the liver and the spleen become additional sites of erythropoiesis, but also some other foci of haematopoietic cells in chickens have been recognized around the spinal cord, thymus, *cloacal bursa*, aorta, heart, pharynx, cranial nerves, spinal ganglia, subcutaneous tissues, muscles, gonads, pancreas, and kidney (ROMANOFF, 1960). In chickens, shortly before hatching and before incorporation of the yolk sac, the erythropoietic tissue migrates into the

skeleton cavities, and the extramedullary erythropoiesis ceases. More extensive haematopoiesis and granulopoiesis have been seen post–hatching in birds other than chicken (BARNES, 1996). The cavities of the skeleton are colonised by red bone marrow at hatching and during the post–hatching period. The postnatal development of erythropoietic tissue has been seen to increase in parallel with the body mass. It has been described in domestic pigeons, European quail (*Coturnix coturnix*) and Budgerigars (*Melopsittacus undulatus*) that when the chicks reach adult mass, the erythropoietic tissue starts decreasing to adult values (STARCK, 1998).

Extramedullary granulopoiesis has been found in the subepithelial tissue of the *cloacal bursa* and in other locations and is most commonly seen at the day of hatching (POPE, 1996).

Following the increasing demand of developing tissues, the embryo produces special haemoglobin with specific affinity for the oxygen inside the egg. It is known that the change of embryonic haemoglobin to neonatal–chick haemoglobin takes place in several steps starting with the lungs breathing air and it can be assumed that some young erythrocytes with different kinds of haemoglobin can be found in peripheral blood. Approximately 14.7% of polychromatic erythrocytes can be seen in the peripheral blood of new born chicks where their number decreases quickly during the first week of life (GLYSTORFF, 1983).

2. 8. 4 Yolk sac reabsorption

The yolk is the main source of nutrients for the chicks after hatching, even though it has already been partially used by the embryo. During the first week of incubation the yolk is completely surrounded by an extraembryonic membrane with absorptive tissue and a dense capillary network, called the yolk sac. This layer is produced by entoblasts, which persist until after hatching, when they start to digest the yolk and to supply the nutrients to the blood (ZIETZSCHMANN and KRÖLLING, 1955). The absorption of nutrients is confined to the surface and vascularized septa (STARCK, 1998). The rest of the yolk sac is incorporated into the embryo's coelomic cavity, getting involved into the intestinal wall shortly before hatching (ZIETZSCHMANN and KRÖLLING, 1955). It is generally considered to be a nutrient reserve for the chicks during the first days of life (KALETA *et al.*, 1994). The blood vessels remain active until

the last vitellin masses (yolk) are used up (ZIETZSCHMANN and KRÖLLING, 1955). The yolk sac is then absorbed during the first week, depending on factors such as the size of the species, the health status, and the nutritional supply. DZOMA and DORRESTEIN (2001) report the normal absorption of the yolk sac in the Ostrich (*Strutio camelus*) to take place within 10 to 14 days post-hatching. Contrary to that, the absorption in chickens takes place in the first 5 days of life (KALETA *et al.*, 1994), and the presence of large quantities of yolk after a week should be considered abnormal in psittacines (CLUBB *et al.*, 1992).

The embryo mainly feeds on albumen during the incubation. The allantochorionic membrane develops villous structures that extend into the albumen. There they produce a fermentative effect, processing the albumen into small pieces that can be absorbed by blood and used by the embryo. Its function stops at hatching (ZIETZSCHMANN and KRÖLLING, 1955).

2. 8. 5 Development of other tissues

It is generally stated that in avian embryos the organogenesis is completed after the first fifth of the incubation time and from then on there is only growth and strong differentiation (BEZZEL and PRINZINGER, 1990).

2. 8. 5. 1 Intestine

The development of the digestive tract in the embryo is faster than that of other systems. This is necessary for achieving optimum digestion and absorption of feed, enabling maximum growth after hatching. It is reported in budgerigars (*Melopsittacus undulatus*) that the absorptive area of the intestine in relation to body weight is at maximum at the time of hatching and decreases when the growing rate diminishes (KLASING, 1999). The intestines of the embryo start working around day 11 of incubation, when it starts swallowing albumen, and at the moment of hatching they are already mature. The glucocorticoids activate the maturation of the intestines for its function of transporting glucose.

In mammals, thyroid hormones and glucocorticoids seem to interact in the development of the intestines and the lung. There is some information supporting a similar situation in birds (McNABB *et al.*, 1998).

2. 8. 5. 2 Lung

During the first days of incubation the oxygen required by the embryo is supplied by the area vasculosa of the yolk sac until day 6, when the chorioallantoid membrane makes contact with the shell and starts exchanging gas by diffusion. At day 8 of incubation, the gas exchanger function of the area vasculosa ceases. At day 12 the chorioallantoid membrane covers the inside of the whole egg shell (TAZAWA and WHITTOW, 2000).

The chicks must change the oxygen supply from an aqueous to an aerial medium at hatching, and the lungs must develop during the nestling period. The functional mechanism of the avian respiratory system differs widely from that of mammals and reptiles, although its development in the early embryonic phases is comparable. The specialisations of the avian lung take place in the last period of the breeding process. One day before hatching in pigeons and 2 – 3 days before hatching in ducks and chickens the main and secondary bronchi including their branchings are completely developed and in the ultimate position. Also all the parabronchi and specific airway nets of the adult birds are already present in number and position before hatching. The circulatory tree is also formed, and the future parabronchi are present as longitudinal tubes with all the smooth muscles in position. Also the atria are in place (DUNCKER, 1983).

When the embryo exerts its internal pipping, the amnion is aerated and the animal starts with regular respiratory movements. At this moment the remnants of amnion fluid in the lungs are reabsorbed by the parabronchi and developing atria (DUNCKER, 1983). Following the internal pipping, the lungs push the amnion proteinaceous fluid out of the future airways to make the breathing of air possible (BEZZEL and PRINZINGER, 1990). The corticosterone level increases prior to hatching and triggers the production of pulmonary surfactant (HYLKA and DONNEN, 1983), in order to enable the expulsion of the remnant fluids. Simultaneously, the infundibulum starts spreading from the base of the atria into the loose coat of mesenchymal tissue and from there the first air capillaries grow around the blood capillaries. At the same time the blood

capillaries connect between arterioles and venules. The mesenchymal tissue keeping the space is supplanted from both sides. The first layer of blood / air capillaries develops in the last days of incubation. During this time the chorioallantoid membrane still supplies the oxygen in full, but with the progressing development of the blood / air capillaries the lungs take over the gas exchange of the embryo and decrease the activity of the extraembryonic membranes. Following the processes initiated by the pipping, ventilation with fresh air through the egg shell from the outside is enabled and when the lungs have matured so far that the total gas exchange is possible, the bird hatches (DUNCKER, 1983).

The development of air capillaries is possible in the volume-constant lungs of birds, while they would collapse in volume-changing lungs. The function of the airsacs is to serve as bellows for the lungs, because constant-volume lungs can not unfold at hatching. The gradual change of gas diffusion from the chorioallantoid membrane to gas convective breathing of the lungs is only possible in a hard–shelled egg; otherwise the shell would collapse (DUNCKER, 1983).

After hatching only the net of blood / air capillaries and the parabronchi show proportional growth until they reach the adult size. This developmental pattern is found in all avian species, but there are considerable differences between precocial and altricial birds. In the latter, only an extremely thin layer of blood / air capillaries develops in the wall of the tubular parabronchi in the embryo. The altricials show a strong growth to functional maturity within 3 – 4 weeks, during which there is a significant increase in size of the network of blood capillaries and air capillaries. On the other hand, precocials hatch with a much thicker layer of blood / air capillaries, due to the necessity of the energy household of these birds. They also show a considerable growth of the respiratory system, but far less so than altricial birds (DUNCKER, 1983).

2. 8. 5. 3 Kidney

Avian neonates hatch with immature glomeruli, which do not show many capillary loops and have no real mesangium. In precocial chicks the maturation of the glomeruli takes about 4 weeks (GERLACH, 1964). Therefore, at least the same time should be allowed for the altricial parrots.

2. 8. 5. 4 Central nervous system

The CNS is immature at hatching and large quantities of granular cells can be seen under the meninges (RANDALL and REECE, 1996). Approximately fifty per cent of the nerve cells undergo apoptosis under normal conditions. Some altricial taxa, such as song birds (passeriformes) and parrots (psittaciformes), hatch with relatively small, underdeveloped brains and exhibit a considerable increase in brain volume after hatching (STARCK, 1989). As mentioned before, the thyroid hormones play an important role in the development of the cytoarchitecture on which brain function is dependent (McNABB and KING, 1993). It has been reported that in hypothyroidism the availability of T_3 for the brain is maintained in order to protect the development of this critical tissue. Two pathways have been noted for reaching this purpose: the increase in the extrathyroidal production of T_3 and the decrease in the degradation of this hormone (RUDAS *et al.*, 1993).

2. 9 AIMS OF THIS STUDY

The high mortality of embryos and chicks occurring between the last days of incubation and one month old has captured the attention of the veterinary staff responsible for parrot pediatrics in Loro Parque. The histopathological results attributed the main part of the deaths to the diagnosis “energy deficiency”.

Although no previous studies have been carried out on the GB of psittaciformes, the accidental finding of empty GBs in most of the dead neonates and embryos has never been described before in the literature of other species. In this study, the presence of empty GBs has been evaluated together with the histopathological lesions in other organs to detect a potential relationship between them. The possible link between the status of the GB and the energy deficiency diagnosis could shed new light on the function of the GB and could be helpful in the diagnosis of energy deficiency. It could also be useful for finding out the real main causes of energy deficiency, thus decreasing embryonic and neonatal mortality. This study is the first documented study of the GB in psittaciformes.

3 MATERIAL AND METHODS

3. 1 MATERIAL

The study was carried out at the Loro Parque Fundación (Tenerife, Canary Islands, Spain), which houses the world's largest parrot collection. At the time, this zoological park lodged 3 447 psittaciformes of 348 different species and subspecies, many of which bred regularly.

Samples were collected during the 2003-breeding season, between the 1st of January and the 31st of October. All dead parrot chicks up to one month of age that were suitable for histopathology were included in a list as possible cases for the study. One bird with a severe stunting problem at day 35 of life was also added as an exceptionally interesting case, although it exceeded the age limit. All species and subspecies which had one or more dead nestlings of the right age were included. Afterwards, a selection had to be made and just the chicks in which the GB could be cut and studied by histopathology were included in the database. Finally, the number of birds investigated was 110.

The database comprised birds of three different sources:

- Baby station (BS): The dead hand-reared birds from the nursery formed the main part of the examination. N= 67
- Nests: Although parent-reared nestlings were plentiful they only contributed a minority. N= 10
- Dead-in-shell embryos (DIS): Embryos found dead in the last period of incubation. These were collected mainly from the incubator when checking the non-hatched eggs. The birds that died during the hatching process were also included as DIS. N= 33

The nestlings in the nursery were carefully weighed each morning to assess a good growth rate and health status. The park veterinarians inspected the baby station daily. All ill chicks were

treated in an appropriate manner. Cloacal swabs for bacteriology and sensitivity testing were cultured to ensure optimum treatment.

The classification of the nestlings by genus as well as by their source can be found in Table 1.

Table 1: Distribution of Genera by Source of Examined Birds

Genus	Origin			Total
	BS	Nest	DIS	
Amazona	6	0	9	**15**
Ara	12	1	11	**24**
Aratinga	4	1	0	**5**
Cacatua	1	0	2	**3**
Cyclopsitta	0	1	0	**1**
Deroptyus	1	0	0	**1**
Diopsittaca	9	0	1	**10**
Eclectus	2	0	0	**2**
Eolophus	1	0	1	**2**
Eos	1	0	0	**1**
Forpus	0	0	1	**1**
Loriculus	0	2	0	**2**
Neophema	0	1	0	**1**
Nymphicus	0	1	0	**1**
Orthopsittaca	0	0	1	**1**
Pionites	2	0	1	**3**
Pionus	4	0	0	**4**
Poicephalus	8	0	1	**9**
Primolius	7	0	2	**9**
Psittacula	2	2	0	**4**
Psittacus	1	0	0	**1**
Pyrrhura	1	0	1	**2**
Rhynchopsitta	1	0	0	**1**
Trichoglossus	4	0	1	**5**
Triclaria	0	1	1	**2**
Total	**67**	**10**	**33**	**110**

BS: Baby station (Hand-reared birds)

DIS: Dead-in-shell

The scientific names of the chick species which formed the database, their common English names and their classification by adult size can be found in Table 2.

Table 2: Scientific and Common English Names and Classification by Adult Body Size

Species, subspecies Scientific name	**Species, subspecies** English name	**Size**
Amazona amazonica	Orange - winged Amazon	M
Amazona auropalliata	Yellow - naped Amazon	M
Amazona autumnalis salvini	Red - lored Amazon	M
Amazona barbadensis	Yellow - shouldered Amazon	M
Amazona ochrocephala nattereri	Yellow - crowned Amazon	M
Amazona oratrix tresmariae	Yellow - headed Amazon	M
Amazona pretrei	Red - spectacled Amazon	M
Amazona rhodocorytha	Red - browned Amazon	M
Amazona ventralis	Hispaniolan Amazon	M
Amazona vinacea	Vinaceous Amazon	M
Amazona xanthops	Yellow - faced Amazon	M
Ara ararauna	Blue - and - yellow Macaw	L
Ara glaucogularis	Blue - throated Macaw	L
Ara macao	Scarlet Macaw	L
Ara rubrogenys	Red - fronted Macaw	M
Ara severus	Chestnut - fronted Macaw	M
Aratinga solstitialis	Sun Parakeet	S
Cacatua leadbeateri	Major Mitchell's Cockatoo	M
Cacatua pastinator	Western Corella	L
Cacatua sulphurea citrinocristata	Citron - crested Cockatoo	M
Cyclopsitta diophthalma	Double - eyed Fig – parrot	S
Deroptyus accipitrinus fuscifrons	Red - fan Parrot	M
Diopsittaca nobilis cumanensis	Red - shouldered Macaw	S
Diopsittaca nobilis nobilis	Red - shouldered Macaw	S
Eclectus roratus roratus	Eclectus Parrot	M
Eclectus roratus solomonensis	Salomon - Eclectus Parrot	M
Eolophus roseicapilla	Galah	M
Eos squamata riciniata	Violet - necked Lory	S
Forpus passerinus	Green - rumped Parrotlet	S
Loriculus vernalis	Vernal Hanging – Parrot	S

Table 2 (continued): Scientific and Common English Names and Classification by Adult Body Size

Species, subspecies Scientific name	**Species, subspecies** English name	**Size**
Neophema splendida	Scarlet - chested Parrot	S
Nymphicus hollandicus	Cockatiel	S
Orthopsittaca manilata	Red - bellied Macaw	M
Pionites leucogaster leucogaster	White - bellied Parrot	S
Pionus tumultuosus	Speckle - faced Parrot	M
Poicephalus robustus fuscicollis	Brown - necked Parrot	M
Poicephalus rufiventris	Red - bellied Parrot	S
Primolius auricollis	Yellow - collared Macaw	M
Primolius couloni	Blue - headed Macaw	M
Primolius maracana	Blue - winged Macaw	M
Psittacula alexandri abbotti	Red - breasted Parakeet	S
Psittacula cyanocephala	Plum - headed Parakeet	S
Psittacula krameri manillensis	Rose - ringed Parakeet	S
Psittacus erithacus timneh	Grey Parrot	M
Pyrrhura lepida	Pearly Parakeet	S
Pyrrhura perlata	Crimson - bellied Parakeet	S
Rhynchopsitta pachyrhyncha	Thick - billed Parrot	M
Trichoglossus capistratus	Rainbow Lorikeet (Edward's Lorikeet)	S
Trichoglossus haematodus caeruleiceps	Rainbow Lorikeet	S
Trichoglossus rubritorquis	Red - collared Lorikeet	S
Triclaria malachitacea	Blue - bellied Parrot	M

Scientific name reference: DICKINSON, 2003

English name and weight reference: HOYO, ELLIOT and SARGATAL, 1997

Size: Small (< 200 g) ; M: Medium (200 g - 600 g) ; L: Large (> 600 g)

In the tables, the code listed in the category “Code of origin” is the reference of the cage where the parents of the nestlings had been accommodated. Consequently, chicks with the same cage reference were siblings.

At necropsy, samples from all the organs and tissues found were collected for the histopathological study, but due to small size and autolysis not all of them could be cut and examined under the microscope. In Table 3 can be found the organs / tissues examined according to the source of the embryos / chicks.

Table 3: Organs / Tissues Examined According to the Source of the Samples

Organ	BS	Nest	DIS	Total
Glycogen Body	67	10	33	110
Thyroid Gland	51	8	20	79
Yolk sac	39	4	33	76
Oesophagus - crop	49	7	19	75
Proventriculus	57	9	20	86
Ventriculus	63	10	30	103
Intestines	65	10	31	106
Liver	66	10	31	107
Pancreas	53	8	7	68
Lung	60	9	29	98
Air Sacs	67	10	33	110
Kidney	66	10	28	104
Heart	66	7	27	100
Thymus	23	1	4	28
Bursa	51	6	9	66
Spleen	44	5	7	56
Bone Marrow	64	10	31	105
Cerebrum	61	10	26	97
Spinal Cord	65	10	32	107
Parathyroid Glands	26	3	4	33
Adrenal Glands	14	2	2	18
Gonads	6	2	1	9
Musculoskeletal System	67	10	33	110
Skin	67	10	33	110
Total	1257	181	523	1961

3. 2 METHODS

3. 2. 1 External examination

The age, species, code of origin and source of each dead chick were noted down in an individual standard post–mortem form. The clinical history and signs prior to death, described by the keepers, the veterinarian or the researcher during the regular visits to the nursery were also illustrated in the individual report. Baby station dead chicks, at their arrival to the clinic, went through a complete external physical examination to evaluate possible outer lesions and the autolytic status of the bird. The skin, eyes, ears, nostrils, beak, oral cavity, choana, feathering stage, crop status, neck, wings and legs, cloaca, umbilicus, body condition and hydration status were all evaluated in this procedure. The weight and the external findings were recorded in the post–mortem form for the final evaluation. The chicks coming from the nests underwent the same protocol, but no clinical signs could be reported in their necropsy record.

The body weight of the nestlings was evaluated (normal vs. stunted) by comparison, using the database of the baby station (see appendix Table 6). This database contained the daily weights of healthy growing chicks reared in the nursery during the last four years. The source of the chicks is also listed in Table 6.

During the regular check of the non–hatched eggs, the embryos found dead during the last period of incubation suitable for the histopathological evaluation, were submitted to an external examination, and their results were noted together with the code of origin and species. The dead embryos were not weighed.

3. 2. 2 Post-mortem examination

The dead chicks underwent a complete necropsy as soon as possible and the post-mortem form (LATIMER and RAKICH, 1994) was completed for each case. If possible, in large chicks, samples of the heart and liver (and also from other organs / tissues if deemed necessary) were

taken for microbiology in a sterile Petri plate. Samples of all organs were fixed in 10% buffered formalin. For very small species (body weight under 5 g) the coelomic cavity and the skull were opened and the whole chick was fixed. All abnormalities were recorded after examination, together with the possible clinical findings, and used to establish a preliminary diagnosis.

The lumbar spinal cord was fixed *in toto* in order to retrieve the glycogen body. After two days of fixation the spinal cord was cut between the third lumbar and first sacral vertebrae with a scalpel blade in order to extirpate the sample. The GB portion was kept in an eppendorf with formalin until its processing.

3. 2. 3 Bacteriological and fungal evaluation

The samples collected for microbiology were processed in the Loro Parque's clinic laboratory. The organs were handled and cut with alcohol sterilized forceps and scissors. With the sterile forceps, the organs were flamed (to eliminate all the surface contaminant bacteria) and then with the sterile scissors, they were cut in two half pieces, one of which was used for culturing the media. The forceps and scissors were sterilized each time before processing each organ. Two kinds of medium were used for the isolation of bacteria and one for fungal organisms:

- Columbia Agar with 5% sheep blood (bioMerieux®);
- MacConkey Agar (bioMerieux®);
- Albicans ID2 Agar (bioMerieux®), a chromogenic medium selective for yeasts that allows the direct identification of *Candida albicans*. The base was Sabouraud's agar which also supports the growth of other fungi.

The cultured plates were incubated in the oven (Memmert®) at 37.7ºC. The bacteriological plates were read at 24 and 48 h. of incubation, and the fungal plates were also read at 72 h. All the results were recorded in the post–mortem form.

In some cases, the histopathological study revealed the presence of bacteria in some organs and tissues. The PAS-stain was also useful for identifying fungal hyphae if present. The bacterial results found by post-mortem microbiology or by histopathology were evaluated together with the recorded clinical signs, treatment, and histopathological findings in order to determine the possible role of the micro-organisms in the death of the nestling.

Occasionally, ancillary tests were done when deemed necessary (cytology, polymerase chain reaction (PCR) for the detection of virus).

3. 2. 4 Histological examination

Fixed samples were processed by a private laboratory[2] for paraffin embedding, cutting, and staining. Three different types of staining were used:

- Turnbull's blue, for iron detection in the liver and occasionally in other organs (kidney, spleen, intestines).

- Periodic acid–Schiff (PAS) reaction, for the detection of polysaccharides, neutral mucopolysaccharides, muco- and glycoproteins, glycolipids, unsaturated fatty acids and phospholipids. A positive reaction was indicated by a bright red colour, which enabled the examiner – amongst other things – to evaluate the filling status of the thyroid glands and the glycogen body.

- The standard stain for histopathology, haematoxylin-eosin (HE) stain.

The slides were studied under the microscope (Leitz Laborlux S.®) at 10, 40 and 100 magnifications, and then discussed with Prof. Dr. Dr. H. Gerlach.

In order to provide a basis for the evaluation of the physiological maturity of tissues and the timeframe for the use of the yolk sac – in addition to the literature cited – comparisons on the

[2] Labor für Tierpathologie Dr. E. von Bomhard, Hartelstr. 30, 80689 Munich

histological picture between nestlings of the same species at the same age were carried out. Because small birds develop more quickly than large ones, the assessments were correlated to the size of the adult birds. They are summarised in Table 4.

Table 4: Estimated Normal Age of Maturity of the Organs, Ceasing of Extramedullary Haematopoiesis, Yolk Sac Use and Absorption

	SIZE OF PARROTS		
	Small	**Medium**	**Large**
	Average Time (days of life)		
Yolk sac completely reabsorbed[A, B]	5	7	8
Begin digesting yolk[C]	0	0	0
End of physiologic fatty liver[D]	8	10	10
Completely developed lung[D]	8 to 14	14 to 21	28
Mature glomerula[E]	28	28	28
Well colonized thymus[D]	3	5	7
Well colonized *cloacal bursa*[D]	5	7	9
Well colonized spleen[D]	7	7	7
Well colonized bone marrow[D]	-1	1	2
End of extramedullary erythropoiesis[D]	7	9	9
End of extramedullary granulopoiesis in spleen[D]	1	2	2
End of extramedullary granulopoiesis in kidney[D]	7	9	9
End of extramedullary granulopoiesis in liver[D]	7	9	9
End of extramedullary granulopoiesis in *cloacal bursa*[D]	1	2	2
Mature cerebrum[D]	20	20	20

[A] CLUBB *et al.*, 1992 [B] DZOMA and DORRESTEIN, 2001 [C] ZIETZSCHMANN and KRÖLLING, 1955 [D] Prof. Gerlach experience and researcher discussions after the histological study of the chicks [E] GERLACH, 1964

All organs / tissues were examined to diagnose the cause of death of the chick, the possible involvement of the GB in it and to find out the possible relationship between the histolopathological lesions and the GB status. For the conclusions drawn from the histopathological examinations, the standard technical terminology and the following definitions (according to the literature) were applied:

3. 2. 4. 1 General terms

► Autolysis – To evaluate autolytic stages, erythrocytes were used in the HE stain. Tissues were considered suitable for histopathology when erythrocytes looked normal or had only a little brownish discoloration in the cytoplasm and had a complete nucleus.

► Not examined – All organs which were not found, not collected or not cut during the histological processing were grouped under this category.

3. 2. 4. 2 Glycogen body

The architecture of the GB, the GB cells structure, the neighbouring structures and the vascularization were studied with the HE stain. The PAS-stain was used to study the filling status of the GB cells, and the structure was classified according to the following definitions:

► Normal – GB with astrocytes almost completely filled with glycogen derivatives.

► Hypotrophic – GB with small particles of PAS-positive material (glycogen derivatives), mainly along the cell membranes of the astrocytes.

► Empty – GB with no PAS-positive material in empty cells.

3. 2. 4. 3 Thyroid gland

The HE stain was necessary for the study of the structure, but the PAS-stain was used for the evaluation of the colloid filling status of the TG follicles. The TG were classified according the following definitions:

► Quiescent status – A normal status of the thyroid gland was assumed when the follicles were entirely filled with colloid. Small vesicles at the follicular epithelium and few half empty follicles were considered an indication of a balanced use of colloid.

► Hypothyroidism – The thyroid follicles presented a cuboidal to cylindrical epithelium, and the majority of the follicles were partially empty or in the process of being emptied.

► Athyroidism – Grouped under this term were TG with cuboidal to cylindrical epithelium and completely empty follicles or follicles with very few remnants of colloid.

3. 2. 4. 4 Yolk sac

The yolk sac was stained using the PAS-reaction in order to evaluate its status as empty, existent only in remnants, moderately filled, or full. Also other classifications were used:

► Broken – During the necropsy some of the yolk sacs were accidentally broken, so their filling status could not be evaluated on histopathology.

► Empty – The presence of small remnants of the yolk sac or the lack of it at necropsy was considered as a reabsorbed / empty yolk sac.

► Early use of the yolk – This classification was made according to the age of the bird, the filling status of the yolk sac, the fatty liver status, and the presence / absence of yolk in the intestines. The embryo uses part of the yolk from the beginning of incubation by absorption of yolk via the blood capillaries. The digestion of the yolk in the intestines via the omphalomesenteric duct normally only begins at hatching. For this reason, the presence of yolk in the intestines before hatching was considered an early use of the yolk.

► Retarded yolk sac – All cases where the yolk sac was still present, although the age of the nestlings exceeded the norm as stated in Table 4, or was more filled than expected.

3. 2. 4. 5 Fatty liver

The iron status of the liver (normal, haemosiderosis, haemochromatosis) was evaluated with the Turnbull's blue stain, together with the HE stain. The fatty liver and other hepatic lesions

(hepatitis, hepatosis, necrosis, anemia, etc.) were described with the HE stain. Other terms used were the following:

► Physiological fatty liver – This fatty liver develops during the first days of life from the lipids stored in the yolk sac (NOBLE *et al*., 1988). It reaches its maximum degree between days 4 and 6, after which the stored lipids are extracted and the liver gets back to its normal state by a certain age as listed in Table 4.

► No or poor fatty liver – No or only small fat vacuoles in the hepatocytes or not in many hepatocytes in relation to the age.

► Retarded fatty liver – Fatty livers up to 8 – 10 days of life (see Table 4).

3. 2. 4. 6 Gastrointestinal tract and pancreas

The gastrointestinal tract was examined under the Turnbull's blue stain for the occasional finding of iron in the intestines, but the main stain for its evaluation was the HE. The pancreas was also studied with the HE stain.

As reported in the literature (see page 31), the intestines should be fully functional at hatching and should reach maximum absorptive capacity during the first few days of life. This means that the surface of the intestinal villi has to be optimal at that time, and the enterocytes must be ready to absorb as much as possible. Therefore, extremely short villi, especially in the duodenum, were considered immature.

3. 2. 4. 7 Lung

The lesions of the respiratory system (pneumonia, feed aspiration, airsacculitis, etc.) were found with the HE stain, helped by the PAS-stain for occasional findings (fluids, fungi, food particles, etc.).

The developmental status of the lungs was evaluated with the HE stain in view of the blood / air capillary layers, the age of the nestling, and the estimated normal age of maturity of these organs (see Table 4).

3. 2. 4. 8 Kidney

The kidneys were studied with the three different stains, the Turnbull's blue for the possible presence of iron in the tubular cells (as seen in iron intoxications), the PAS for the detection of membranous glomerulopathies and the HE for the report of other lesions (tubulonephrosis, tubulonecrosis, immature glomeruli, etc.). Terms used not widely reported in the literature are described as follows:

► Immature renal glomeruli – Glomeruli which were generally small, shrunken in appearance, not showing many open capillary loops, and not yet with a distinct mesangium. It seemed that the glomeruli only matured one by one.

► PAS–positive membranous glomerulopathy – Besides other possible causes, this was recognised as a sign of Avian Polyomavirus, where antigen–antibody complexes are deposited in the subendothelium of the capillary loops. Thickened capillary walls in the glomeruli could be observed using PAS–stain (GERLACH *et al.*, 1998). A demonstration of the virus by PCR was necessary for the diagnosis.

3. 2. 4. 9 Lymphatic system

When examined, the thymus, the *cloacal bursa* and the spleen were evaluated according to species, age and estimated maturation of the tissues (see Table 4). For their study the HE stain was used.

► Involution of the *cloacal bursa* was diagnosed by the proliferation of the epithelium within the follicles between the cortex and the medulla, together with degenerating precursor cells.

3. 2. 4. 10 Haematopoiesis

Extramedullary haematopoiesis is the main source of erythrocytes and granulocytes during embryonic life. In small parrot species the bone marrow colonisation starts shortly before hatching and in the large ones after hatching (pers. comm., Prof. Gerlach). The extra- and intramedullary haematopoiesis was examined in the HE stain and classified under these terms:

► Extramedullary haematopoiesis – Presence of active haematopoietic cells out of the bone marrow, between the connective tissue of other organs / tissues.

► Retarded extramedullary haematopoiesis - Retarded extramedullary erythropoiesis and / or granulopoiesis was determined by the presence of extramedullary haematopoietic tissue after the estimated dates in Table 4.

► Immature erythrocytes / anaemia – The oxygen supply was found to be insufficient due to two different erythropoietic disturbances:

- Low numbers of erythrocytes;
- Immature erythrocytes (reticulocytes) with elongated net–like nuclei and a lack of haemoglobin, appearing bluish in the HE stain, and earlier forms in the peripheral blood with still round nuclei (polychromatic erythrocytes), derived from the bone marrow (see Table 4).

3. 2. 4. 11 Brain maturation

The spinal cord, brain and its maturation status were studied on the HE stain. Some lesions were found (neuropil vacuolation, neuronal vacuolar degeneration, anaemia, autolytic, etc.) but another term was also introduced for the maturity status:

► Immaturity of the brain – This condition was diagnosed in chicks of approximately 3 weeks of age, which still showed too many nerve cells and / or a thick outer granular layer (sometimes still many layers) in the cerebrum and / or cerebellum.

3. 2. 5 Calculations

A brief numerical approach was performed for some organs, to numerically estimate the evolution of the lesions with the age of the bird, and try to find a correlation between them. This analysis was performed applying a numerical value to each lesion and calculating the average for each age. This average numbers were represented graphically to see the trajectory of the lesions when a chick grows. No variance analysis was performed to evaluate if the results were significant.

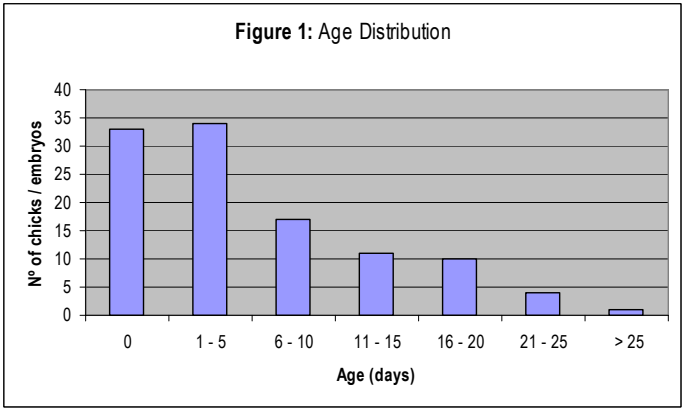
4 RESULTS

4. 1 DATABASE DESCRIPTION

All embryos and dead chicks up to one month of age were collected, but only those fresh enough were included in the list of possible cases. The chicks and embryos, in which the GB was not cut, were also removed from the database of the study. In the end it contained 110 cases of dead chicks and embryos that had died in the last period of incubation. The age distribution of the database is listed in Table 5 and represented in Figure 1.

Table 5: Age Distribution of the Chicks / Embryos of the Database

Age (days)	No. of chicks / embryos
0	33
1 - 5	34
6 - 10	17
11 - 15	11
16 - 20	10
21 - 25	4
> 25	1
TOTAL	**110**



The weight of the birds was compared with the available data of normal growth from the baby station (see appendix Table 6) in order to evaluate whether or not a chick was stunted. Nestlings for which no reference weights were found were classified according to the experience of the examiners or by using data from closely related species (see appendix Table 6). Just 18 (16.4%) birds presented a normal weight (± 10% the average weight), 43 (39.1%) were found under the average weight and 16 (14.5%) were above its average. In all age groups, the main part of the chicks (≥50%) was under the normal weight, as can be seen in Table 7.

Table 7: Weight Evaluation of the Chicks / Embryos According to the Age Groups

Age (days)	Weight				TOTAL
	Under normal	Normal	Above normal	Unknown	
0	1	1	1	30	**33**
1 - 5	15	10	6	3	**34**
6 - 10	12	2	3	0	**17**
11 - 15	6	2	3	0	**11**
16 - 20	6	3	1	0	**10**
21 - 25	2	0	2	0	**4**
> 25	1	0	0	0	**1**
TOTAL	**43**	**18**	**16**	**33**	**110**

Regarding the source, the main part of the chicks came from the BS (60.9%), where they could be easily controlled and kept in the refrigerator when dead. Although many chicks were found in the nests, they just represent the 9.1% of the samples, because most of them were autolytic. The rest of the database (30%) were chicks dead during the hatching process and embryos dead in the last period of incubation when were found not to be autolytic (see Table 1).

Not all organs could be studied because, in some cases, they were not found during the necropsy, or they were not cut when the slides were prepared (see Table 3).

The clinical pictures seen in the BS chicks was: sudden death during the first week of life and after one week stunting problems with dry and pale skin, disproportioned body, globose head, recurrent infections, delayed opening of the eyes, poor feathering, delayed emptying of the crop, etc.

All histopathological results are summarised in Table 28 in the appendix.

4. 2 GROSS ANATOMY OF THE GLYCOGEN BODY

The GB was found to be present in the spinal cord of psittacine chicks and embryos as described in other species. It was localized inside the vertebral column between the third lumbar and first sacral vertebrae, lying in the middle of the nervous tissue, in the sinus called *Fossa rhomboidea spinalis*. It was found to be uncolored, oval shaped (in a dorso-ventral view) and had a gelatinous-like consistency. The GB could be easily identified with the naked eye as a separated structure different from the spinal cord. The GB was surrounded cranially, ventrally and caudally by the spinal cord, while dorsally, it was protected by the roof of the vertebral column.

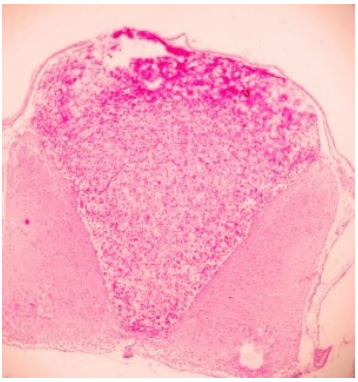
The size of the GB depended on the bird's size. Due to its small size, no macrosopical information concerning the vascularization, innervation and other related structures could be reported.

4. 3 HISTOLOGIC STRUCTURE OF THE NORMAL GLYCOGEN BODY

To study the GB, transversal sections of the sacral spinal cord were used, and it could be stated that the structure presents an inverted triangular shape. The connection between the two halves of the spinal cord was seen to be reduced to a small ventral area. In some cases a meningeal layer could be identified covering dorsally the GB. The GB at optical microscopy was mainly composed by a single kind of cell, large in size and with an irregular polygonal shape. The nuclei and perinuclear cytoplasma were displaced to the edge of the cell due to the presence of an empty-appearing structure - when seen in the HE stain - occupying the main part of the cell. This structure, which is supposed to be the glycogen derivatives stores, stained bright red in the PAS-stain, confirming this assumption (see Picture 2). No connections could be seen between the GB cells and the neighbouring tissues.

The central canal passed through the GB in its most ventral portion, without interposition of a basement membrane between the ependymal and GB cells. A rich vascularization was evident

and the GB cells were in direct contact with the basal lamina of the vessels. No evidence of innervation inside the GB was seen.



Picture 2: Normal GB – Astroglial cells filled with glycogen derivatives (PAS – stain, 10X)

The term “normal GB” (according to the filling status of the cells) used as reference in this thesis was taken from what is described in the literature: “The GB cells contain large amounts of glycogen derivatives stored”. In this study empty GB cells were more frequently seen.

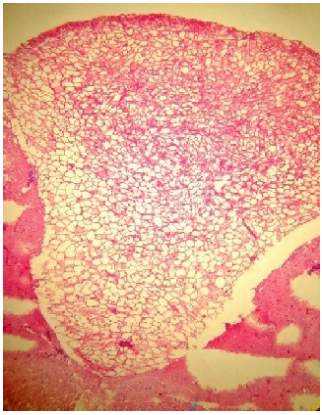
The GB was examined in 110 cases, out of which only 7 (6.36%) had a normal filling of the GB cells (full - slightly hypotrophic GB) and 11 (10%) showed moderate filling (moderately hypotrophic GB) (see appendix Table 8).

4. 4 PATHOLOGICAL STRUCTURES

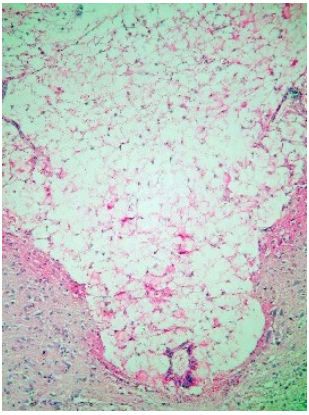
4. 4. 1 Glycogen body

From the 110 cases, 55 (50%) showed severe hypotrophy and 37 (33.6%) empty GB (see appendix Table 8). In these cases, the GB cells were found almost or completely empty of glycogen derivatives, respectively (see Picture 3 and 4). The nuclei and cytoplasma continued to

appear at the edge of the GB cells, and the rest of the space was empty. Even when studied with the PAS-stain, no material or just very few remnants of glycogen derivatives were seen filling the space. No PAS-positive material, suggesting glycogen derivatives, were seen either outside the cells or in the tissue nearby the GB. The small blood capillaries were present as in the normal GB, and no PAS-positive material could be reported inside of them. Neither were pathologic changes described in the central canal and ependymal cells.



Picture 3: Hypotrophic GB – Astroglial cells partially filled with glycogen derivatives (PAS stain, 20 X)

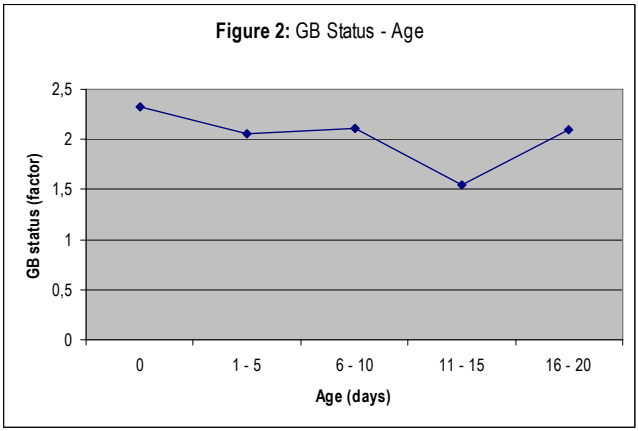


Picture 4: Empty GB – Astroglial cells almost empty of glycogen derivatives (PAS stain, 20 X)

An empty GB was seen more frequently in embryos, while severe hypotrophy of the GB had a higher incidence in chicks of 1 – 5 days of age (see Table 9 and Figure 2). The GB status did not seem to correlate with age, as can be seen in Figure 2. In this figure, no birds older than 20 days were included because the GB of only 5 birds was considered not representative for those age groups.

Table 9: Glycogen Body Status According to the Age of the Chicks / Embryos

Age (days)	Empty GB	Severely hypotrophic GB	Moderately hypotrophic GB	Full (slightly hypotrophic) GB	TOTAL	Mean score*
0	18	10	3	2	**33**	77 / 33 = **2.33**
1 - 5	6	24	4	0	**34**	70 / 34 = **2.05**
6 - 10	5	10	1	1	**17**	36 / 17 = **2.11**
11 - 15	3	4	0	4	**11**	17 / 11 = **1.54**
16 - 20	3	5	2	0	**10**	21 / 10 = **2.10**
21 - 25	2	1	1	0	**4**	9 / 4 = **2.25**
> 25	0	1	0	0	**1**	2 / 1 = **2.00**
TOTAL	**37**	**55**	**11**	**7**	**110**	
Factor	3	2	1	0		



*Mean score = [(No.Empty GB * 3) + (No.Sev.Hypotr.GB * 2) + (No.Mod.Hypotr.GB * 1) + (No.Full GB * 0)] / No.Total Age Interval

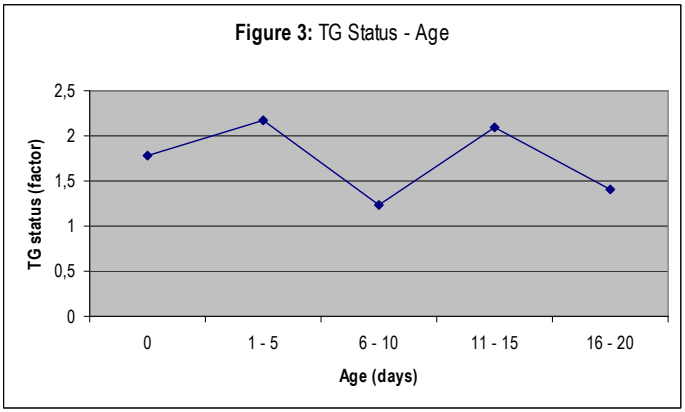
4. 4. 2 Thyroid gland

The thyroid glands (TG) were studied in 79 nestlings, and more than half of them (69.6%) presented histopathological findings in the TG compatible with athyroidism. Thirteen birds (16.4%) were diagnosed with hypothyroidism, and the thyroid gland displayed a quiescent stage in 11 (13.9%) cases only (see appendix Table 8). Athyroidism appeared mainly in embryos and 1 – 5 day old chicks (see Table 10 and Figure 3). In Figure 3 just birds between 0 and 20 days old were included, because 5 birds were not a considerable amount to represent the two remaining age groups.

Table 10: Thyroid Gland Status According to the Age of the Chicks / Embryos

Age (days)	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid not examined	TOTAL	Mean score♦
0	19	1	0	13	**33**	59 / 33 = **1.78**
1 – 5	22	3	2	7	**34**	74 / 34 = **2.17**
6 – 10	3	4	4	6	**17**	21 / 17 = **1.23**
11 – 15	6	2	1	2	**11**	23 / 11 = **2.09**
16 – 20	3	1	3	3	**10**	14 / 10 = **1.40**
21 – 25	1	2	1	0	**4**	8 / 4 = **2.00**
> 25	1	0	0	0	**1**	3 / 1 = **3.00**
TOTAL	**55**	**13**	**11**	**31**	**110**	
Factor	3	2	1	0		

♦Mean score = [(No.Athyr. * 3) + (No.Hypothyr. * 2) + (No.Quiescent TG * 1) + (No.TG not exam. * 0)] / No.Total Age Interval



4. 4. 3 Yolk sac and fatty liver

Concerning the fat stores of the embryos and chicks just 32 (29.1%) birds made a correct use of them (see appendix Table 12), reabsorving the yolk sac, storing the fat in the liver (physiologically fatty liver) and finally using this fat in the estimated times (Table 4).

Of all the yolk sac findings, the most frequent was its early use before hatching (33.6%), which can be diagnosed in embryos and chicks younger than 5 days old. A retarded yolk sac was present in 11 of the nestlings, and it was detected in chicks older than 3 days with large yolk sacs or older birds with remnants of it (see Table 11 and appendix Table 12).

In 40 (36.4%) cases the birds were judged not to have a proper physiologically fatty liver in the light of their age and filling status of the yolk sac (see appendix Table 12). In the category “No proper physiologically fatty liver”, 21 birds younger than 8 days presented a poor or non-existent physiologically fatty liver that should be there. On the other side, the 19 birds older than 8 days of age classified under this term (see appendix Table 12), presented a moderate – heavy fatty liver which according to the estimated ages of Table 4, should not be there.

Table 11: Yolk Sac and Fatty Liver Findings According to the Age of the Chicks / Embryos

Age (days)	Early use of the yolk sac	Retarded yolk sac	No proper physiologically fatty liver
0	29	0	0
1 - 5	8	2	20
6 - 10	0	3	2
11 - 15	0	4	9
16 - 20	0	2	6
21 - 25	0	0	3
TOTAL	**37**	**11**	**40**

4. 4. 4 Gastrointestinal tract and pancreas

When the upper gastrointestinal tract was examined, no obvious lesions were found except in one case, a *Pionus tumultuosus* (ID No. 77) that showed a purulent, necrotising pharyngitis. It can be supposed that this pharyngitis caused the aspiration pneumonia that was also present in the bird (see appendix Table 16).

The 86 examined proventriculi were all normal, except in one bird which had suffered chronic proventriculitis (*Amazona oratrix tresmariae*, ID No. 8).

Ventriculi were studied in 103 cases; they were mainly normal only showing lesions in the following birds:

- *Amazona vinacea* (ID No. 13): petechiae in the muscular layer
- *Ara ararauna* (ID No. 18) and *Ara glaucogularis* (ID No. 25): oedema around the chief cells
- *Eclectus roratus solomonensis* (ID No. 61): anaemia
- *Primolius couloni* (ID No. 88): erosions in the koilin layer caused by bacteria and yeasts and infiltrations of inflammatory cells
- *Primolius couloni* (ID No. 89 and 90, siblings): abnormal mucosa (cyst–like structures in the mucosa filled with koilin)

The examination of the intestines was mostly performed in the duodenum, because this was the intestinal part fixed for histopathology together with the pancreas. If abnormalities were noted in other sections of the intestine, samples of those parts where also taken for the histological investigation. In this study, it was observed that the intestines easily became autolytic. Of the evaluable cases, 5 intestines were immature and 16 displayed oedema of the villi (see appendix Table 13). Intussusception was confirmed in an *Ara glaucogularis* (ID No. 27). The presence of iron in the enterocytes of *Trichoglossus haematous caeruleiceps* (ID No. 107) is explained later (see page 84). Other lesions found are listed in Table 13 (in the appendix).

The histopathological examinations of the pancreas revealed 16 nestlings with a complete lack, or only remnants of zymogenic granules, in the acinar cells. Two animals showed degeneration of endocrine cells suggesting possible diabetes mellitus (see appendix Table 14). In 3 cases degeneration of exocrine cells was seen.

4. 4. 5 Liver

The liver was examined in 107 cases, 23 of which were autolytic and 27 (25.2%) normal. The remainder of the chicks presented one or more lesions which are classified in order of frequency and summarised below (see also appendix Table 15):

- No proper fatty liver: 21 cases (19.6%)

- Retarded fatty liver: 19 cases (17.7%)
- Fatty liver degeneration: 10 cases (9.3%)
- Necrosis of liver: 9 cases (8.4%)
- Hepatosis: 8 cases (7.5%)
- Anaemic liver: 4 cases (3.7%)
- Haemosiderosis (hepatocytes appear normal): 3 cases (2.8%)
- Haemochromatosis (lesions of hepatocytes): 2 cases (1.9%)
- Hepatitis: 1 case (0.9%)
- Other findings: see appendix Table 15

All the siblings of *Primolius couloni* (ID No. 89, 90 and 91) from cage LV 671 displayed a retarded fatty liver and fatty liver degeneration, and all of them died within the third week of age. Two also showed an abnormality of their ventricular mucosa (reported before on page 60).

In two cases, storage of glycogen granules inside the hepatocytes was detected at an early age. The animals concerned were a *Diopsittaca nobilis nobilis* (ID No. 57) and a *Primolius auricollis* (ID No. 87).

Since both gluconeogenesis and lipogenesis in birds mainly take place in the liver, this organ was examined with special care. Poor or delayed extramedullary haematopoiesis was noted, even if the liver itself appeared to be normal. In the cases of hepatosis, usually no inflammatory reaction was observed except in a few birds, where inflammatory cells were present in higher numbers than normal. This was interpreted as a reaction to the degeneration and not as an inflammation *per se*.

4. 4. 6 Respiratory tract

No obvious lesions were found in the upper respiratory airways, except in one case (*Diopsittaca nobilis nobilis,* ID No.59), where a mycotic purulent inflammation of the nasal cavity was seen. The hyphae were described as Aspergillus–like.

The trachea itself presented no obvious lesions, and only in 6 cases were feed particles found inside. These could have been artefacts produced during the necropsy, or caused by recent feed aspiration before dying (see appendix Table 16).

Pneumonia was diagnosed in 7 cases, 2 (28.6%) of which were due to feed aspiration. In 5 chicks feed aspiration in the lungs was observed without pneumonia being present, suggesting sudden death of the birds by asphyxiation and oxygen deficiency. Feeding techniques in hand–fed chicks or weakness induced by illness could have been the causes for feed aspiration (see appendix Table 16).

In two chicks of 3 days of age, an *Amazona oratrix tresmariae* (ID No. 8), and a *Primolius maracana* (ID No. 95), fluid in the parabronchi and airsacs was seen respectively. This is normal in embryos and 1 day old chicks, but at day 3 after hatching the airways should be free of fluids.

The development of the lung is necessary for gas exchange and energy metabolism. Evaluating the maturity status of the lungs was difficult because of the variances between individuals and between species. In this investigation the lungs of 98 birds were examined, out of which 21 (21.4%) had underdeveloped lungs in relation to their age, 9 (9.2%) were too autolytic to be evaluated and the remainder (69.4%) were normal (see Table 17 and appendix Table 6).

Table 17: Development Status of the Lung According to the Age of the Chicks / Embryos

Age (days)	Lung				TOTAL
	Underdeveloped	Normal development	Autolytic	Not examined	
0	0	20	9	4	**33**
1 - 5	2	28	0	4	**34**
6 - 10	5	8	0	4	**17**
11 - 15	7	4	0	0	**11**
16 - 20	6	4	0	0	**10**
21 - 25	1	3	0	0	**4**
> 25	0	1	0	0	**1**
TOTAL	**21**	**68**	**9**	**12**	**110**

4. 4. 7 Kidney

The kidney of the chicken chick takes about 4 weeks to mature before reaching its optimum performance. In this investigation, kidneys showing retarded immature glomeruli were seen just in 1 case (*Trichoglossus rubritorquis* ID. No. 108), in which the bird was 35 days old. Immature glomeruli were diagnosed in relation to the age of the bird together with the appearance of the glomeruli (see literature page 33).

The lesions most frequently seen in the kidneys were degeneration of renal tubules, with 17 (16.3%) animals displaying a tubulonephrosis. Quick autolysis is characteristic for renal tubules and accordingly, could be seen in many cases. Forty–five (43.3%) kidneys were considered normal. Chronic tubulonephrosis was reported in 9 (8.6%) cases, 4 of which were seen in the two subspecies of *Diopsittaca nobilis* and 2 in *Poicephalus rufiventris*. Some other findings were as follows (see also appendix Table 18):

- Anaemic kidneys: 9 cases
- Glomerulonephritis: 4 cases
- PAS–positive glomerulopathies: 1 case
- Tubulonecrosis: 2 cases

The PAS–positive glomerulopathy was present in one bird *Deroptyus accipitrinus fuscifrons* (ID No. 49), which was 19 days old. PCR-testing for APV was performed, and a negative result was obtained.

4. 4. 8 Heart

With 21 cases (21%), degeneration of the myocardium was the heart lesion most frequently seen, followed by 14 chicks (14%) with interstitial oedema between the heart muscle fibers. The first condition was present in 4 out of 8 *Ara ararauna,* originating from two pairs.

Myocarditis was diagnosed in four cases, and the only other lesion found in the heart was a thin left ventricle wall in an *Eclectus roratus roratus* (ID No. 60) (see appendix Table 19).

4. 4. 9 Haematopoiesis

Extramedullary erythropoiesis was mostly reported in the liver, while granulopoiesis was seen in the kidney, the liver, and to a lesser degree in the spleen (see appendix Table 20). Sometimes, haematopoiesis around the spinal cord was also seen in 1–2 day old chicks after which time it started migrating into the bone. Colonisation with haematopoietic tissue always seemed to start in the skull bones. 24 and 38 cases of delayed extramedullary erythropoiesis and granulopoiesis were reported, respectively. In 24 cases delayed extramedullary haematopoiesis was found, 14 nestlings displayed only delayed extramedullary granulopoiesis. Regarding the species distribution of delayed haematopoiesis (see appendix Table 20), the condition can be seen more frequently in small–sized species such as *Psittacula spp., Poicephalus rufiventris*, and *Aratinga solsitialis,* and also in one medium–sized species, *Primolius couloni*.

The bone marrow was examined in 105 chicks, and 23 (21.9%) of these were diagnosed with poor bone marrow colonisation. Only 36.4% of the birds with delayed extramedullary erythropoiesis in which the bone marrow was examined showed poor bone marrow colonisation. Similar results were obtained in birds with delayed extramedullary granulopoiesis and whose bone marrow was examined.

Increased numbers of immature erythrocytes in the peripheral blood were reported in 6 cases. Out of these, 4 also presented delayed extramedullary erythropoiesis and granulopoiesis (see appendix Table 20).

Poor erythropoiesis was suspected in two cases (*Deropytus accipitrinus fuscifrons,* ID No. 49; and *Trichoglossus haematodus caeruleiceps,* ID No. 107), in which the liver showed moderate to profound iron deposits in the hepatocytes and Kupffer cells. The intestines of the latter bird also revealed the presence of iron in enterocytes and in the middle of the villi.

4. 4. 10 Central nervous system

The cerebrum was studied in 97 cases and anaemia was reported in 18 (18.5%) of these. An immature brain was seen in 7 birds (7.2%), neuropil vacuolation in 7 (7.2%), and neuronal vacuolar degeneration in 1 (see appendix Table 21).

Two unusual lesions in the cerebrum were found in the following birds:

- *Amazona xanthops* (ID No. 15): Hypoxic degeneration. This condition is caused by ependymal cells producing a glycoprotein which blocks the cilia of the surface of the cells, thus inhibiting the movement of the fluid in the ventricular system. This lesion seems to have a genetic background (pers. comm., Prof. Schmahl).
- *Rhynchopsitta pachyrhyncha* (ID No. 103): Haemorrhages around the arteries in the cerebrum. These presented a congenital dysplasia since the endothelium had not

developed, and therefore the vessels were not intact. A genetic background could also be suspected (pers. comm., Prof. Schamhl).

The cerebellum could be examined only in 13 cases, and abnormalities were found in 4 (30.8%) of them:

- Immature cerebellum and focal neuronal vacuolar degeneration in *Poicephalus robustus fuscicollis* (ID No. 78).
- Immature cerebellum was also reported in two *Primolius couloni* (ID No. 90 and 91). The last one, together with a *Loriculus vernalis* (ID No. 67) apart from the immaturity, also presented focal neuropil vacuolation.

The spinal cord was examined in 107 animals and neuropil vacuolation was the main lesion observed (35.5%). Only in 5 cases, a neuronal vacuolar degeneration was diagnosed (see appendix Table 21).

4. 4. 11 Bacterial evaluation

Following microbiological examination, bacteria were found in 51 (46.4%) of the birds. Analysing all the data, the role of the bacteria in the death of the nestlings was categorised as follows (see appendix Table 22):

- 21 chicks suffered from septicaemia (19.1%)
- 7 chicks had local infections (6.4%)
- 5 chicks showed secondary infections (4.5%)

Table 23: Bacteriological Status of the Chicks / Embryos According to their Age

Age (days)	Bacterial status			
	Local infection	Secondary inf.	Septicaemia	Not relevant
0	0	1	0	0
1 - 5	2	0	10	6
6 - 10	1	1	4	8
11 - 15	1	2	2	3
16 - 20	2	1	3	1
21 - 25	1	0	2	0
> 25	0	0	0	0
TOTAL	7	5	21	18

Escherichia coli was the bacterium most frequently isolated. In some cases the presence of bacteria was not considered as relevant, either due to their low numbers or to their known low pathogenicity, or simply because they were considered to be part of the normal flora.

4. 4. 12 Other infectious agents

Although fungi can also be the cause of health problems, this was less frequently the case in the present study than problems following bacterial infection. The few cases found are listed as follows:

- Yeasts in the intestinal lumen of three birds, *Ara ararauna* (ID No. 23), P*ionus menstruus* (ID No. 74) and *Poicephalus rufiventris* (ID No. 80), which were considered local colonisations. In these cases, yeasts had probably been in the feed.
- An *Ara macao* embryo (ID No. 34) showed yolk sac mycosis which was considered to be a complicating agent that may have grown through the egg shell. The primary findings in this case were athyroidism and an empty GB.

- In the liver and lungs of a *Primolius couloni* (ID No. 90) fungi were found following cytological examination. Since distinctive fungal lesions were neither seen in the lungs nor in the liver following histopathology, no particular attention was paid to this.
- Mycotic purulent inflammation of the nasal cavity in *Diopsittaca nobilis nobilis* (ID No. 59)(see page 62).

Parasites in the intestines were only found in one case in a chick coming from the nest (*Neophema splendida*, ID No. 68). The worm larvae were identified as nematodes. This bird also showed a poorly colonised spleen with some necrotic areas.

4. 4. 13 Lymphatic organs

The thymus could be studied in 28 birds, all of which showed good colonisation (see appendix Table 24). The thymus seems to be colonised earlier than the *cloacal bursa* and spleen.

The *cloacal bursa* of 66 nestlings was examined, and poor colonisation with precursor cells was observed in 15 (22.7%) birds. In 9 (13.6%) chicks, the *cloacal bursa* was well colonised with precursor cells, but almost no mature lymphocytes were present (see appendix Table 24). Other relevant findings in the *cloacal bursa* were as follows:

- Premature involution of the *cloacal bursa* in 2 birds: *Eclectus roratus roratus* (ID No. 60) and *Eolophus roseicapilla* (ID No. 63). In the latter two, many bacteria were found in the histopathological examination (see appendix Table 22). This finding supported the suspicion of septicaemia despite the fact that in both cases the thymus showed a good colonisation.
- In another case of septicaemia (*Deroptyus accipitrinus fuscifrons,* ID No. 49) the lymphocytes of the medullar *cloacal bursa* were necrotic.

Out of 56 examined spleens, 22 (39.3%) were poorly colonised with precursor cells. Six spleens were colonised with precursor cells, but not many mature lymphocytes were recognisable (see appendix Table 24). Myelocytomatosis was seen in the spleen of one bird (*Amazona*

amazonica, ID No. 3). Its thymus and the cloacal bursa were not examined. The liver of the bird showed extensive amounts of perivascular cuffing with myelocytes.

4. 4. 14 Other organs

In some cases other organs, structures or glands were examined, or only isolated lesions were seen (see appendix Table 25). They are listed as follows:

Parathyroid glands: Degeneration of water–clear cells in a *Pionus tumultuosus* (ID No. 75).

Ultimobranchial body: in contrast to phasianiformes, in parrots – at least in the species examined in this study – this structure was surrounded by a capsule. No lesions reported.

Musculoskeletal system:

- 2 cases of metabolic bone disease: *Nymphicus hollandicus* (ID No. 69) and *Primolius couloni* (ID No. 91).
- 1 case of poor bone mineralisation: *Diopsittaca nobilis nobilis* (ID No. 59).
- 1 case of myodegeneration: *Primolius couloni* (ID No. 89).

Skin:

- Subcutaneous oedema, possibly as a sign of athyroidism: *Ara ararauna* (ID No. 17)
- Focal purulent dermatitis: *Aratinga solstitialis* ID No. 41)

The testes were examined in some cases, and none of them presented pathological lesions. The ovaries were also studied in a few cases, and the only reported lesion was a premature follicular atresia in a *Nymphicus hollandicus* (ID No. 69).

4. 5 INTERRELATIONSHIP BETWEEN NORMAL GLYCOGEN BODY AND OTHER ORGANS / STRUCTURES

Only 16.4% of the birds in this study presented a moderately hypotrophic or normal GB, so it was difficult to compare such small group with the main group presenting a pathologic GB. The great variability of the study (different ages, different species and subspecies, etc.) together with the reduced number of normal GB's found, and the lack of controls, made difficult to find any relationship between the normal GB and any other organ. Any possible link between normal GB and any other findings would not be trustworthy.

4. 6 INTERRELATIONSHIP BETWEEN PATHOLOGICAL GLYCOGEN BODY AND OTHER ORGANS / STRUCTURES

No true relationship between the pathological GB and any other organ could be stated in this investigation, due to great variability of the database and the lack of controls for comparison. Nevertheless, the most common findings in chicks with empty / hypotrophic GB and its possible relationship with the GB will be reported below.

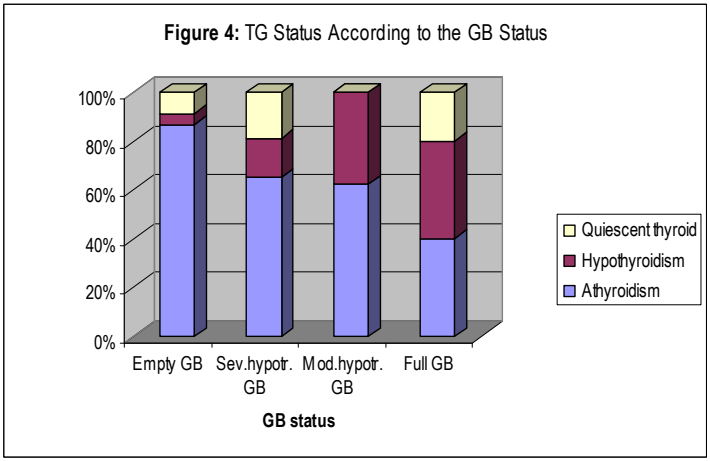
4. 6. 1 Thyroid gland

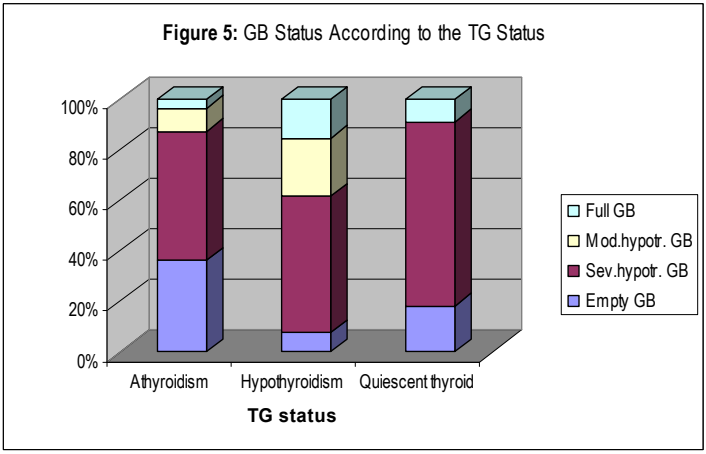
A high number of birds with athyroidism were found to have an empty or hypotrophic GB. The GB in 48 (87.3%) out of 55 chicks displaying athyroidism was almost or totally void of glycogen derivatives (see Table 26 and Figures 4 and 5). The incidence of athyroidism seemed to decrease when the amount of glycogen derivatives inside the GB increases (see Figure 4), although this could be due to a lower number of birds representing the moderately hypotrophic and normal GB.

Table 26: Glycogen Body Status Related with the Thyroid Gland Status of the Chicks / Embryos

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid not examined	TOTAL
Empty GB	20	1	2	14	**37**
Sev. hypotr.	28	7	8	12	**55**
Mod. hypotr.	5	3	0	3	**11**
Full GB	2	2	1	2	**7**
TOTAL	**55**	**13**	**11**	**31**	**110**

Figure 4: TG Status According to the GB Status





4. 6. 2 Yolk sac and fatty liver

The early use of the yolk sac was diagnosed in 37 cases, all of them suffered from athyroidism / hypothyroidism and all but one (*Ara macao*, ID No. 31) from empty / hypotrophic GB (see appendix Table 28).

According to the literature, one cause of retarded yolk sac could be bacterial infections. In this investigation, five of the ten birds concerned were found to be infected with bacteria (two displaying septicaemia and three a local infection of the yolk sac). Eight of these ten birds had an empty or hypotrophic GB, whereas 7 out of 9 showed athyroidism or hypothyroidism.

All the birds with a non proper physiologically fatty liver presented also an empty / hypotrophic GB.

4. 6. 3 Gastrointestinal tract

The five birds presenting immature intestinal villi had an empty / hypotrophic GB. Four out of five of these birds also showed athyroidism, and one showed hypothyroidism.

4. 6. 4 Respiratory tract

A direct relationship between the GB status and lung development does not seem to exist.

A high incidence (80%) of underdeveloped lungs occurred in one small species, *Aratinga solsitialis* (see Table 6).The animals of this species originated from three different pairs (2 chicks from LV 171, 1 chick from LV 173 and 1 chick from LV 170). It was obvious that immaturity of the lungs was more frequent in stunted, underweight birds than in nestlings with normal weight: out of 21 chicks with underdeveloped lungs the weights of 18 (85.7%) were below normal (see Table 27).

Table 27: Development Status of the Lung According to the Weight Evaluation of the Chicks / Embryos

	Lung				
Weight	**Underdeveloped**	**Normal development**	**Autolytic**	**Not examined**	**TOTAL**
Under normal	18	19	0	6	**43**
Normal	0	17	0	2	**19**
Above normal	3	13	0	0	**16**
Unknown	0	19	9	4	**32**
TOTAL	**21**	**68**	**9**	**12**	**110**

4. 6. 5 Central nervous system

The birds with an immature cerebrum all presented an empty / hypotrophic GB, except one (*Poicephalus rufiventris*, ID No. 84) with a moderate filling of the structure. No relationship seems feasible.

One bird (*Poicephalus robustus fuscicollis*, ID No. 78) was found with an immature cerebrum and a quiescent thyroid gland, as opposed to 5 birds that were found with an immature cerebrum and athyroidism or hypothyroidism.

5 DISCUSSION

5.1 DATABASE DESCRIPTION

The age distribution of the studied group can not be related to the status of the GB because the database was performed according to the presence / absence of GB cuttings in the evaluated slides. It can only be noted that there is a bigger number of samples in the groups of late embryonic deaths (0 days of age) and 1 - 5 days of age. The bigger amount of cases in these two groups is probably linked to the fact that the mortality is higher in these periods. The greater mortality in embryos and young chicks is commonly related to the lack of fully developed immune systems making them more sensible to infectious agents (FLAMMER and CLUBB, 1994). More dead embryos / chicks gives additional probabilities for cutting the GB during the histological preparation, and a higher representation in the database on these categories. Also more reliable results are obtained in these groups due to the bigger amount of samples. As the chick grows, the mortality decreases due to a more resistant system (subcutaneous fat stores for energetic reservoir and thermal insulation, acquisition of thermoregulatory ability, feathering, etc.) and a completely developed immunological system.

The weights of the embryos have not been precisely evaluated for several reasons: the lack of a reference weight in each embryonic stage for each species, the presence of embryonic membranes and fluids distorting the real weight, and lack of knowledge of the death date (not the aim of the study). Considering these factors, precise weighing of the embryos before necropsy is not considered valid. On the other hand, the chick weights have been evaluated to the extent possible and it can be seen that 54.5% were under the normal weight. This statement is quiet logical considering that the database is composed of dead chicks that died mainly under pathological situations.

Concerning the source of the chicks, just few of them came from the nests because they were often found to be autolytic. This is due to the fact that the nests were not controlled daily to avoid

unduly disturbing the breeding pairs. On the other side, the majority representation of chicks from the nursery comes from a greater control and easier accessibility.

The clinical picture of stunting commonly observed in this study has been previously described by other authors (CLUBB *et al.*, 1992), and is related to problems of inadequate husbandry, underlying diseases, poor incubation techniques, etc. The low growth rates and stunting problems have been frequently linked to poor and unbalanced diets (especially low calorific intakes) (CLUBB *et al.*, 1992).

5. 2 GROSS ANATOMY OF THE GLYCOGEN BODY

The localization and macroscopical description of the GB (shape, appearance, etc.) reported in the results concides with the main literature (WATTERSON, 1949; WELSCH and WÄCHTLER, 1969; LYSER, 1973; MÖLLER and KUMMER, 2003).

The GB vascularization and innervation of the psittacine chicks have not been described in this study because it is not one of the aims and also because special and expensive techniques are required. Also the small size of the vessels and nerves affording the structure makes difficult the macroscopical description.

5. 3 HISTOLOGICAL STRUCTURE OF THE NORMAL GLYCOGEN BODY

The main descriptive features reported in the results of the transversal section of the spinal cord at the level of the GB agree with the ones noted by WATTERSON in 1949. Occasionally a meningeal layer could be identified dorsally to the GB. Specific stainings must be used for the proper evaluation of these structures; this could explain the fact of describing the meninges in few cases. Otherwise, the meningeal localisation of the GB has been previously described by DICKSON and MILLEN (1957) and was not the purpose of this study.

The histological picture of the GB cells and the vascularization described in the results agree with the ones of LYSER (1973). As in this study, her examinations also report the presence of an empty appearing area occupying the main part of the GB cells. According to DE GENNARO (1959), it is confirmed that under the PAS – stain this area turned bright pink - red in normal GBs.

The kind of stains used and the low microscope magnification in this examination were not adequate to study the connections between the GB cells described by other scientists (LYSER, 1973). The innervations between the GB cells described by different authors in the literature (WELSCH and WÄCHTLER, 1969; PAUL, 1971; SANSONE and LEBEDA, 1976; DE GENNARO and BENZO, 1987) has not been observed in the histological examinations of the present study, probably due to the small size of the nervous fibres, the low microscope magnification and the use of inadequate stains.

5. 4 PATHOLOGICAL STRUCTURES

5. 4. 1 Glycogen body

In this study, only 6.4% of the studied GBs presented cells completely filled with glycogen - derivatives. No reference about empty or hypotrophic GBs have been found in the literature for any species, while in the project 93.6% of the GB cells were partially or completely empty of glycogen - derivatives.

Most of the available literature about the GB deals with chickens, quails, and ducks, which are all precocial species. Few studies have been conducted in altricial species, using pigeons (WELSCH and WÄCHTLER, 1969). No investigations have been carried out so far in psittaciformes (altricial species) and one question should be solved first: do the parrot species hatch with the GB filled with glycogen derivatives? Avian embryogenic development in a number of species from penguins to songbirds has been described for the same 42 normal stages (RICKLEFS and STARCKS, 1998), and the amount of energy required is approximately the same (BEZZEL and PRINZINGER, 1990), independent of the mode of development at hatching. The differences between altricial and precocial birds are attributed to a longer phase of tissue

maturation in precocials, and differences in the duration of the embryonic stages at the end of the incubation period (RICKLEFS and STARCKS, 1998). The GB appears at 7.5 – 7.75 days of incubation (stage 31) in chickens, and even the first astroglial cells of the GB already contain granules of glycogen. They start accumulating more glycogen steadily from stage 36 onward (day 10 of incubation). From these prerequisites it can be concluded that the GB of altricial birds at hatching must contain at least a reasonable quantity of glycogen derivatives filling the astroglial cells. Supporting this theory, in the investigation two dead macaw embryos in the last stage of incubation (*Ara ararauna* ID No. 17 and *Ara macao* ID No. 31) presented a GB reasonably well filled with glycogen derivatives.

Glycogen is the storage form of glucose. It consists of 1,4-glycosidic bonds and at a distance of 3 to 12 glycosyl subunits also of 1,6–glycosidic bindings resulting in branching chains and clusters forming particles of 10 – 40 nm in the cytoplasm (MERTENS, 1996; WIESNER and RIBBECK, 2000). The water solubility of glycogen is probably directly related to the branching and bonds between the glucose molecules. Depending on the binding and branching between the glucose molecules, the solubility of the glycogen derivatives diluted into the water while fixing and staining the samples could be variable. The question “Could the solubility of the glycogen derivatives have been the cause of finding empty / hypotrophic GBs?” remains open.

The presence of small blood vessels in an empty GB is a sign of recent emptying of the structure; otherwise, if the structure had been empty for a long time, the small capillaries would no longer be there because of their rapid regression. This observation suggests possible emptying of the GB as an emergency, survival mechanism of the body. It is known that the GB cannot release glucose directly into the blood because of its lack of glucose–6–phosphatase (BENZO *et al.*, 1975). Therefore, the emptying pathway is not known, but the glycogenolytic pathway producing lactate, pyruvate and ketone bodies, as suggested by BENZO and DE GENNARO (1981), could be a possibility.

The GB has been found empty or hypotrophic in most of the chicks which have been investigated. However, this finding does not appear to be related to the species, age or source of the nestlings. Although there appears to be a higher frequency in some genera like Amazona, Ara, Dipositta ca, Primolius and related genera (Psittacula, Aratinga), the reproductive success

was lower in other genera (lower numbers of embryos and chicks). Moreover, an empty or hypotrophic GB has been found in a wide variety of psittaciforme species and subspecies, and all of the seven chicks displaying a normal GB belong to different species. In view of their age, birds presenting an empty GB range from late dead embryos to up to one month old chicks (see Table 9 and Figure 2 in page 56. Note that in figure 2, birds older than 20 days are not included because there were only 5).

5. 4. 2 Thyroid gland

The thyroid glands have been studied in precocial and altricial chicks to learn which role they play in the different developmental stages of the bird at hatching and post–hatching. In this context, two different patterns have been described according to the endothermic response to cooling (McNABB and collaborators, 1984, 1985, 1988, 1996, 1997). In altricial birds, the activity of the TG is low at hatching, and they do not present a peak in their thyroid hormone–levels at the time of hatching, as is typical for the precocial species. The activity of the TG in altricials is low during the first eight days after hatching, and the TH concentration inside the follicles is also low. Thyroid stimulating hormone (TSH) in nestling doves becomes detectable in the pituitary gland at 4–6 days of life, and coincides with the gradual increase of TH in the TG. The TG of embryonic doves has been reported to not respond to external supplementation of TSH (McNICHOLS and McNABB, 1988). In the Red–winged Blackbird (*Agelaius phoeniceus*), an altricial species, OLSON *et al*. (1999) describe increasing thickness of the follicular epithelium (cuboidal–cylindrical) in the TG until day 6, and then a decrease for the rest of the nestling period. These authors also measured the mean area of the follicles and describe an increase up to day 8 of life, by which time it reaches its maximum size and then starts to decrease. According to these findings, a histological picture of the thyroid gland in altricial species emerges, but this should probably not be generalised.

According to the literature (McNABB and CHENG, 1985; OLSON *et al.* 1999), the TG in altricial hatchlings is physiologically empty and the colloid starts filling up the follicles during the first week of life, reaching adult levels by up to 2 weeks of age. The results mentioned refer to the Ring Dove (*Streptopelia risoria*), the European Starling (*Sturnus vulgaris*) and the Red–winged

Blackbird (*Agelaius phoeniceus*). The embryos and the newly hatched chicks of the psittaciformes investigated here showed not only a cuboidal to cylindrical epithelium but also signs of hypothyroidism such as immaturity of various organs and oedema in the intestinal villi. The cuboidal to cylindrical epithelium indicates a previous challenge by TSH. If there had been an appropriate answer, TH would have been produced, and normal growth of the bird achieved. However, this was not the case. Based on the literature, a TG with an estimated 50% of the follicles being empty during the first week of life is considered physiological. The status of the TG in relation to the age of the birds can be seen in Table 10 and Figure 3 (see page 57 and 58). Even so, the proportion of "normal" TG during the first five days (7.4% of the studied TG) appears to be low in this investigation. The number of quiescent TG was found to increase in the 6 – 10 days old group (36.4%) and the 16 – 20 days old group (42.8%). The decrease in the prevalence of normal TG seen in the sixth category onwards (older than 21 days) can be attributed to the low number of samples in that period. Since psittaciforme nestlings gain weight more quickly under parental care than precocial chicks, they may also need an earlier increase in TH – even more so than doves, starlings, and blackbirds – at the beginning of the growth period. It is possible that psittaciformes require a longer time to fully develop their TG than the other altricial species studied, a fact that could be reflected in the relatively high number of nestlings diagnosed with athyroidism or hypothyroidism in this study.

The occurrence of TG with cuboidal–cylindrical follicular epithelium has been reported in cases of athyroidism and hypothyroidism, respectively, in this investigation. Under the influence of TSH, the TG are stimulated to produce TH. A negative feedback, mediated by T_4 (and also by T_3 in chickens), reduces the release of TSH by the pituitary gland. A hypofunctional TG produces low levels of T_4 that are insufficient to trigger the negative feedback and to reduce the production of TSH. This continuous stimulation of the TG leads to apparent hypertrophy of the follicular epithelium which then acquires a characteristic cuboidal–cylindrical shape (SCHMIDT, 2002). Probably – when the pituitary gland / TG system starts working in altricials – the production of TH during the first days is not sufficient to reach the levels needed to trigger negative feedback, and the continuous stimulation with TSH is the cause of the shape of the epithelium during the first days. During the examinations of the TG, the presence of thick epithelium was a regular finding in most of the organs with incompletely filled or empty follicles at any age. This fact, in addition to

the high number of cases with diagnosed hypothyroidism, gives rise to the suspicion of an additional problem apart from the suspected subclinical biotin deficiency.

It has been demonstrated that egg yolk contains low levels of maternal thyroid hormone which plays a role in the development of many tissues in the embryo during the initial incubation process. The quantity of deposited TH in the egg depends on the levels in the blood of the laying hen (McNABB and WILSON, 1997). SCHMIDT and REAVILL (2002) suggest that hypothyroid hens may lay eggs with low levels of TH, leading to a congenital goiter. The most common cause of acquired hypothyroidism is iodine deficiency, but the fact that this study took place on an island nullifies this possibility. Hypothyroidism has also been seen when plants with goitrogenic substances or with genetically induced biosynthetic problems are fed (SCHMIDT, 2002). In any case it is thought that hens with hypothyroidism will rarely breed. Recent investigations by McNABB (2001) suggest that lipophilic pollutant chemicals such as polychlorinated biphenyls (PCB) accumulate in the feed chain and – competing with maternal TH – get stored in the egg. The author describes possible competitive inhibition at two different levels, one related to the transport of the thyroid hormones from the hen to the egg, and the other at the binding site of thyroid hormone receptors. This interference by PCB with the normal function of thyroid hormone is likely to affect the TG of the embryo and consequently to hamper embryogenesis and also the metabolism of the newly hatched chick. Different psittaciforme species may vary in their susceptibility to inhibiting factors.

The condition of the TG in this examination does not seem to have any special relation to the source, species or genera involved, but the age of the animals appears to play an important role as explained before.

5. 4. 3 Other organs / structures

Bacterial infections could explain half of the cases of retarded yolk sacs in this study, being their main cause according to ROMANOFF (1960). Other possible causes have also been described such as a high incubation temperature and / or humidity, inadequate oxygen levels during the last week of incubation, high energy diets fed to the chicks, lack of exercise by the

nestling (in precocial birds), and particularly oedematous chicks at hatching, which itself can be a sign of athyroidism / hypothyroidism (SPEER, 1996; DZOMA and DORRESTEIN, 2001). Also fibrin or products of purulent inflammation can mechanically occlude the omphalomesenteric duct (GRATZL and KÖHLER, 1968). Although TG or GB problems were noted in many of the birds with retarded yolk sacs, it cannot be ruled out that any of those situations described in the literature could have taken place without being recorded, because it was not a subject of this investigation.

Escherichia coli was the bacterium isolated most often. Its pathogenicity is rather variable but was not investigated since *E. coli* and most of the other enterobacteriaceae were not considered to be a component of the autochthonous intestinal flora (GERLACH, 1994). The handling of the chicks without gloves could have been one of the possible transmission pathways, although flies and other insects can also act as a vector. Another possibility is that the feed was contaminated with some of these bacteria due to inadequate sanitary measures during the process of fabrication. Few random tests were satisfactory.

In some cases the presence of bacteria in the nestlings was not considered relevant due to low numbers or low pathogenicity, or because they were considered to be part of the normal flora. Birds do not have lymph nodes, instead they have lymph follicles spread throughout all organs (except the brain and testes). The presence of lymph follicles in the liver is normal, for the reason that part of this organ's blood supply comes directly via the portal vein from the intestines and usually contains some bacteria. In contrast to mammals, the avian liver is not sterile, and can contain some bacteria as components of the autochthonous flora, but also pathogens (GERLACH, 1994). The interference of the antemortem treatments with antibiotics was also taken into consideration regarding the postmortem microbiologic and histopathologic investigations.

Fatty liver has been reported to occur in some calves when they do not receive sufficient energy to remove the stored fat in the liver (pers. comm., Prof. Dr. Dr. H. W. Rambeck[3]). Might this be the cause of some retarded fatty livers in the chicks of this examination? Retarded fatty livers can be due to energy deficiency because fat needs energy to be metabolized? Also SCHMIDT *et al*. (2003), report that biotin deficiency is one of the causes of hepatic lipidosis.

[3] Ludwig – Maximilians University, Munich

The storage of glycogen granules in hepatocytes seen in a *Diopsittaca nobilis nobilis* (ID No. 57) and a *Primolius couloni* (ID No. 87)) at an early age of 4 and 2 days respectively suggests a glycogen storage disease with a genetic background (pers. comm., Prof. Schmahl).

It is difficult to decide if the complete or partial lack of zymogenic granules in the acinar cells of the pancreas was a pathological finding or only a post–prandial emptiness without the presence of degenerated cells. No answer can be given for the cases seen in the present project, but it is logical to conclude that any exocrinic pancreatic diseases would negatively affect digestion and decrease the absorption of nutrients. Apparently from the observations of this study it can be said that the zymogenic granules (when present) are more resistant to autolysis in birds than in mammals. Accordingly, they have been still visible in cases where the intestines started showing autolysis.

The presence of fluid in the lungs and airsacs is considered physiological in embryos and neonates during the first day of life. Contrary to this, ROMAGNANO *et al.* (2001) report *in ovo* aspiration of amniotic fluid in psittacine chicks seen in histopathology as being responsible for the death of chicks after hatching. These authors noticed an increased incidence in African Grey Parrots (*Psittacus erithacus*) and they decreased the humidity in the incubator as a corrective measure with successful results. It is agreed that the presence of fluids in the respiratory airways in chicks older than one day is considered pathological as seen in *Amazona oratrix tresmariae* (ID No. 8) and *Primolius maracana* (ID No. 95).

Forty four percent of the cases of chronic tubulonephrosis were seen in *Diopsittaca nobilis*. The nestlings concerned originated from 3 different pairs of the two subspecies. Since the lesions were already chronic (partly mineralized), it is likely that the damage occurred during breeding. Experimental confirmation of this assumption is necessary.

Avian Polyomavirus (APV) is known to cause PAS-positive glomerulopathy, but the bird affected in this study was too young and its antibody defence system not sufficiently established to transfer antibody–antigen complexes from the blood to the glomerular capillaries. Accordingly, although PCR-testing for APV was performed, negative results were obtained. Based on the

literature, it was concluded that other pathogenic processes – not yet described in parrots – could have been the cause for this lesion (GERLACH *et al.*, 1998).

Iron in the intestines of the *Trichoglossus haematodus caeruleiceps* (ID No. 107) could have originated from haemorrhages within the upper part of the gastrointestinal tract or from the feed. If iron is only present in the liver (and spleen) it is considered either a sign of non-physiological haemoglobin production (anaemia), a deposit following haemolysis, or the expression of a disturbance of iron metabolism (particularly the destruction of iron–metabolizing cells in the spleen).

Accordingly to the literature (ROMANOFF, 1960; BARNES, 1996; POPE, 1996) the presence of extramedular haematopoiesis in the yolk sac, liver, spleen, around the spinal cord, cloacal bursa and kidney few days post-hatching can be confirmed.

The thymus examined have always been found to be well colonised in comparison to the other lymphocytic organs. Therefore, it appears that the thymus shows mature lymphocytes well before the *cloacal bursa* and the spleen. The explanation might be that the B–cells from the *cloacal bursa* require the presence of T–helper cells to function.

The involution of the *cloacal bursa* in the literature is reported to begin at 9 weeks of age in chickens, at the onset of sexual maturity in small altricial birds (ITCHON and LOWENSTINE, 1997), and in large species it is supposed to take place at 6–9 months of age. This literature reinforces the diagnosis of premature involution of the *cloacal bursa* in the *Eclectus roratus roratus* (ID No. 60) and *Eolophus roseicapilla* (ID No. 63).

The myelocytomatosis described in the spleen of the *Amazona amazonica* (ID No. 3) is suspected to be caused by a retrovirus that requires a special configuration of the histocompatibility complex B to become infective. However, this bird was too young to get infected by the virus, and the presence of tumour-transformed cells around the spinal cord confirmed the fact that it was egg-transmitted. The parents may be asymptomatic carriers of the virus (when infected later in life) and transmit it vertically to the progeny – hence the term egg-transmitted.

Some of the findings of this investigation suggest the possible existence of diseases with a genetic background in the collection of the Loro Parque Fundación:

- Glycogen storage disease
- Diabetes mellitus
- Glycoprotein produced by the ependymal cells
- Congenital dysplasia of the endothelium of blood vessels in the brain
- Myelocytomatosis (because the possible retrovirus requires a specific genetic configuration at the histocompatibility complex–B to become infective)

5. 5 INTERRELATIONSHIP BETWEEN THE GLYCOGEN BODY STATUS AND OTHER ORGANS / STRUCTURES

Not many histopathological lesions seen in this investigation can be related to the condition of the GB, because of the great variability (different species and subspecies, different ages, different disorders, etc.), and the lack of controls available for comparison. Also, there are only 7 chicks with full GB (6.4 %) in the database, and no relevant comparisons can be made between normal and empty / hypotrophic GB–groups. Nevertheless, the most common findings in chicks with empty / hypotrophic GB and the possible relationship with the GB status will be discussed.

There seems to be a correlation between the conditions in the GB and the conditions in the TG, particularly in embryos and 1- 5 days old chicks where the incidence of emptiness and hypotrophy in both structures and athyroidism in the TG is highest (see appendix Tables 29, 30, 31, 32, 33 and 34). Considering the key position of the TG in the energy metabolism, the lack of glycogen derivatives and thyroid hormones may combine to cause the death of the chick. In the age groups older than 5 days the occurrence of athyroidism decreases and more chicks with a quiescent TG are seen. At the same time the prevalence of an empty or hypotrophic GB does not appear to decrease as regularly as seen in the TG. From the literature it is known that the enzyme activity of pyruvate carboxylase (PC) in chickens increases up to day 16 of life and then levels out over a period of up to 30 days (TUSCHY, 1983). The question whether or not the lack

of thyroid hormone (TH) prevents gluconeogenesis cannot be answered following this investigation.

Around the hatching period the main stores of energy for the chick are the yolk sac and the yolk–dependent physiologically fatty liver. Evaluating both of them individually and jointly, energy deficient situations can be conjectured that suggest early use of the yolk sac and / or an incorrect physiologically fatty liver. In most of the chicks in which one of these conditions was seen the GB showed poor or no glycogen content. This fact coincides with the findings of some authors in the literature (TERNI, 1924; DOYLE and WATTERSON, 1949; KUNDU and BOSE, 1974; WELSCH and WÄCHTLER, 1969), who suggest emptying of the GB in energy-demanding situations. However, another possibility must be taken into consideration when working with embryos and neonates: has the glycogen body filled correctly in the first place during embryogenesis? For this investigation it can be stated that most of the studied embryos DIS presented deficiencies in the GB filling, but the question if the GB were correctly filled during embryogenesis or were emptied in an energy-demanding situation (during the incubation) can not be solved here.

The histopathological examinations of stunted chicks revealed the immaturity of several organs that could be explained by the low availability of glucose for the tissues to mature and grow normally, thus delaying their development. For example, where immaturity of the intestinal villi was present, the GB revealed deficiencies in glycogen, too. The same was observed in birds with non-physiologically fatty liver. Immaturity of the brain was seen in 7 cases and in all of them together with an empty / hypotrophic GB. However, a relation between the conditions of these structures cannot be deduced from this investigation.

Retarded extramedullary haematopoiesis could also be considered a sign of immaturity, but a clear relation with the hypotrophic status of the GB cannot be asserted because it was also found in chicks with normal GB. The possibility that this delayed haematopoiesis could be related to athyroidism or hypothyroidism will be discussed below. Immature erythrocytes in the peripheral blood and underdeveloped lungs are also signs of immaturity, but again do not seem to be directly related to the status of the GB, because of their presence in some chicks with normal GB.

The developmental status of the lungs seems to be independent of the condition of the GB because on the one hand, many birds displaying glycogen deficiencies in the GB had normal lungs and, on the other, nestlings with full GB were found to have underdeveloped lungs.

5. 6 INTERRELATIONSHIP BETWEEN THE THYROID GLAND STATUS AND OTHER ORGANS / STRUCTURES

Since none of the chicks with quiescent TG have shown early use of the yolk sac, although some of them had failed to produce a physiologically fatty liver or revealed a retarded fatty liver, the effect of the TH in the yolk / fat stores is uncertain. Due to the importance of TH in the growth and differentiation of tissues, immaturity of some organs would be expected in cases of athyroidism / hypothyroidism, as described in this investigation.

The literature describes maturation effects of TH on the intestines, the lungs, the brain and lymphoid tissues (NUNEZ, 1984; BACHMAN and MASHALY, 1986; McNABB, 1995; McNABB *et al.*, 1998). In accordance with one reference (McNABB *et al.*, 1998), the examinations in this study did not reveal immature intestines in those birds with quiescent TG. Even so, all the birds presenting immature intestines showed also deficiencies in thyroid colloid. As with the GB, the TG status does not seem to be directly related with the developmental status of the lungs: 11 chicks were found to have underdeveloped lungs as well as athyroidism or hypothyroidism, and 3 chicks showed quiescent TG together with underdeveloped lungs. Only preliminary conclusions are possible. An influence of TH on the embryonic genesis of the lungs in accordance with the findings of DECUYPERE (1993) and McNABB *et al.* (1998) is almost certain. The underdevelopment of lungs described here occurs after hatching because of the peculiar growing process of the altricial lungs (see page 32). At this time it is not possible to identify the various factors that influence pulmonary growth. From this investigation, it can be suggested that one of these factors is the size of the chick. From 21 chicks diagnosed with underdeveloped lungs, 85.7% were below normal weight (see Table 27 in page 73). Therefore, it can be suggested that stunted chicks – in an attempt to save energy – develop lungs according to their body size and to their oxygen requirements instead of developing lungs of a size that is not yet necessary. Underdeveloped lungs were prevalent in almost all the birds belonging to two small species in our

database, *i.e. Aratinga solstitialis* and *Psittacula cyanocephala* (see appendix Table 6). Smaller species require more frequent feeding due to the small capacity of their crop in the first days of life. Consequently, they are more prone to stunting (and probably to underdeveloped lungs) if hand–rearing timetables do not cover their requirements. It is also possible that the time required for the total development of the lung is longer than the estimate quoted in Table 4 for small-sized parrots.

From the TH maturition effects on the brain it can be said that out of the cases displaying immature brains, 1 chick had a normal TG, while all others showed athyroidism (57.1%) or hypothyroidism (14.2%) (just in one case the TG were not examined). The high probability of the brain depending on TH for normal development is stated in the literature (McNABB *et al.*, 1998). It is based on the fact that, in chicken embryos, T_3 receptors have been detected in labelled hormone–binding experiments before day 7 of incubation, *i.e.* before the TG is organised into follicles and secretes a significant amount of hormones. The supposed positive effect of the TG on humoral immunity (BACHMAN and MASHALY, 1986) can not be proven, mainly because the chicks included are not old enough for the necessary tests. It is a fact that some of the chicks with quiescent TG also present distinctive underdevelopment in the lymphocytic organs, and most of these chicks also show empty / hypotrophic GB. The cause of these immune deficiencies remains uncertain.

Immature glomeruli have been reported in 1 case (*Trichoglossus rubritorquis* ID No. 108) at 35 days of age, together with immature cerebrum which can be probably associated to the diagnosed athryoidism, although just one case is not representative.

The delayed extramedullary haematopoiesis can be related to athyroidism / hypothyroidism. It is recognized as a sign of immaturity. Although one of the chicks with normal GB and a quiescent TG shows retarded extramedullary haematopoiesis, this does not necessarily eliminate a possible relationship between the TG status and retarded extramedullary haematopoiesis. The possibility of a link cannot be clarified here. Retarded extramedullary haematopoiesis has been seen most often in small–sized species such as *Psittacula spp*., *Poicephalus rufiventris*, *Aratinga solstitialis,* and in one medium–sized species, *Primolius couloni* (see appendix Table 20). The factors controlling termination of the extramedullary production of blood cells are unknown. The

estimation of the normal time scale for extramedullary haematopoiesis (Table 4) does not rule out the possibility that some species may require longer periods. For birds apart from parrots, suppositions have been made ranging from weeks and months (SCHMIDT, 1999). In Prof. Gerlach's experience, renal extramedullary granulocytopoiesis in King Penguins (*Aptenodytes patagonicus*) may persist until one to one and a half years of age.

Energy is central to metabolism and can be produced by different pathways. It is also certain that it depends directly on the correct function of different systems and organs. The failure of one of these systems causes the animal to enter into a progressive cycle of energy deficiency if corrective measures are not taken successfully. The gastrointestinal tract, the respiratory tract and the circulatory system provide the cells with nutrients and oxygen for energy metabolism. Therefore, disorders affecting the functionality of these systems will also affect energy metabolism. Immature intestinal villi and oedematous villi have been reported in the study, and in both situations the absorption of nutrients and the production of energy can be considerably affected. Besides this, other diseases affecting the gastrointestinal tract or the associated organs, such as liver and pancreas, can decrease the digestion of feed and render some nutrients unavailable for absorption. Also, the oxygen supply can be reduced, by two different mechanisms: firstly in cases of underdeveloped lungs or lung diseases decreasing gaseous exchange; and secondly in cases of decreased erythrocyte counts or immature erythrocytes with low haemoglobin levels.

All the situations described above can be primary causes of energy deficiency, but can also be secondary to disorders affecting energy metabolism thus decreasing the speed of maturation of these organs. Examples for such disorders are low biotin levels (explained below) and athyroidism / hypothyroidism. In chicks with subclinical biotin deficiency or TG disorders, lesions affecting the organs described can be contributing factors for the animals entering into a vicious cycle of energy deficiency.

In adult birds, TH have a positive effect on metabolic activity and also increases blood glucose levels by stimulating the release of hepatic glycogen. In the breeding egg and during the first week of life, the levels of TH remain low, suggesting that the use of carbohydrates as energy sources is lower than that of lipids. Obviously, the evolutionary strategies have chosen lipids as

the form of storage for the egg and the neonate bird because the energy obtained through the combustion of lipids (39.3 kJ/g) is higher than the energy obtained from glycogen (17.6 kJ/g) or proteins (18.0 kJ/g) (MERTENS, 1996). With the physiological rise of TH levels during the first weeks of life the use of carbohydrates also increases to cover the high energy demands of the growing tissues and endothermic regulation. This is shown by the increase of gluconeogenic enzymes in the liver of the chick up to day 16 and the following, constant level of activity up until one month of age. The fact that dove nestlings do not need as much TH as parrot chicks could be explained as being physiological, because the neonates of the doves are fed for approximately the first week of their lives with crop milk, an excretion which is almost free of carbohydrates. Only from then on are they increasingly fed with carbohydrates. Lipolysis, glycogenolysis and glucose obtained through gluconeogenesis supply the necessary energy to maintain the high metabolic rates and tissue growth. In this critical period, all of these pathways need to function optimally in order to supply the required energy. In this study, the supposed low levels of biotin (see below) combined with athyroidism / hypothyroidism could have caused a decrease in gluconeogenic activity, low levels of glucose in blood, and decreased metabolic activity, leading the chick into a vicious cycle of energy deficiency. This could explain the simultaneous appearance of an empty / hypotrophic GB and energy deficiency signs, which have been often seen in the nestlings in the study.

5. 7 ROLE OF BIOTIN IN THE FILLING STATUS OF THE GLYCOGEN BODY

During incubation gluconeogenesis must take place in the embryo's metabolism to enable it to synthesize glucose from lipids and proteins. This pathway is the only possible source of glucose for the embryo from day eight of incubation, when almost all the carbohydrates in the egg have already been exhausted (TUSCHY, 1983). When the astrocytes of the GB start storing large amounts of glycogen derivatives from day ten of incubation, they need glucose for synthesis, which is generated by gluconeogenesis in the liver. The hydrolysis of lipids produces glycerol and free fatty acids, which are transported – bound to albumin – in the blood to the liver. Here, they are activated by coupling with coenzyme A and then degraded by β–oxidation to acetyl–CoA–derivatives that can enter the citric acid cycle. The glycerol is converted in the liver and kidney to dihydroxyacetone phosphate, which can in turn readily be converted into glucose (MERTENS,

1996; WIESNER and RIBBECK, 2000). Proteins are lysed into amino acids including the so–called glucogenic amino acids such as alanine, glycine, cysteine, serine, tryptophan, aspartic acid, threonine, phenylanaline, isoleucine, methionine, valine, glutamic acid, histidine, proline and arginine. Glucogenic amino acids can be degraded into C_4–dicarboxylic acids or to pyruvate, and the whole carbon structure, or at least parts of it, can be used for gluconeogenesis. Intermediate products are, besides pyruvate, oxaloacetate, α–ketoglutarate and succinyl–CoA. The substrates of proteins and lipids are present in the egg in abundance as are the necessary enzymes for gluconeogenesis. Biotin may be one of the possible limiting factors for this pathway.

Subclinical deficiencies of biotin in the breeding hens can be the cause of low concentrations of biotin in the eggs laid. The early embryonic deaths reported in cases of biotin deficiency (KLASING, 2000) can be completely avoided with low concentrations of this vitamin, because the eggs will start developing normally. The late embryonic deaths and the deformities observed in biotin–deficient eggs (KLASING, 2000) could be minimal or even absent in eggs with low biotin contents that, albeit low, do not drop to the status of a truly biotin–deficient egg. An embryo with low levels of biotin can probably develop normally and even hatch with no clinical signs while stores of the vitamin are still available. Once biotin has been used up and the stores are empty, some embryos may die in the late incubation period and some chicks may die after hatching or even later when the biotin requirements for growing increase. Besides this, PEARCE and BALNAVE (1978) note that biotin deficiency affects PC to a higher degree than the other biotin–dependent enzymes. In an egg with a low biotin concentration or in a chick with subclinical biotin deficiency, PC would be the first enzyme to be affected and a reduction of gluconeogenesis due to the decrease of this enzyme's activity would be the first symptom to occur. This would lead to a reduction of blood glucose levels and to reduced glucose availability in the tissues. In this situation the astroglial cells of the GB could not store as much glycogen as normally expected, leading to a GB with a partial or total lack of glycogen derivatives. At this point the embryo might be forced to start using the stores intended for the neonatal period – such as the yolk sac – before hatching. This would lead to early use of the yolk sac and / or to a low physiologically fatty liver. All these hypotheses can be deduced from the findings in this study, but they would have to be proved experimentally.

The role of avidin in biotin availability for the chick is uncertain. WHITE *et al.* (1992) noted that biotin bound to avidin is available to the chick when it swallows the albumen at the time of hatching. However, the same authors (WHITE *et al.*, 1992) report that adult birds eating raw eggs are unable to use biotin bound to avidin, because the complex is very resistant to digestion. No explanation is given on how the complex is degraded by the chick to release the biotin. Another investigator (DAVIES, 2000) describes the presence of high levels of avidin in the yolk sac of newly hatched chicks. This binds the biotin of the nestling leading to reduced gluconeogenesis. If the latter hypothesis is true, yolk sac-derived avidin could be deadly in subclinically biotin–deficient neonates. This fact could explain the sudden deaths seen during the first week of life.

After the first week of life, having used up their pool of biotin, chicks might start showing deficiency symptoms if they have not been supplemented with biotin via the feed. The clinical picture of reduced gluconeogenesis could be manifested as stunting in the nestlings, and this has been frequently observed in the chicks from the nursery. Otherwise, the differential diagnosis of this clinical picture should also include athyroidism and hypothyroidism, conditions for which stunting is a typical symptom.

Concluding from this study it can be said that the GB in normal conditions is full of glycogen derivatives at hatching in psittaciforme species. Problems in embryogenesis interfering with the gluconeogenesis of the embryonic liver, such as low biotin content in the egg, can decrease the storage of glycogen inside the astroglial cells of the GB. An empty GB can be indicative of low biotin content during the embryonic and / or neonatal period together with other histopathological findings reported in this study (early use of the yolk sac, non–physiological fatty liver, delayed maturation of some tissues, etc.). The emptying of the GB prior to death is also likely to occur by an unknown pathway in an effort by the bird to survive. The TG of newly hatched psittaciforme birds seems to present low activity or none at all, which is considered pathological. The interference of PCB in the TG / TH system seems to be a reasonable explanation of the high number of cases with athyroidism / hypothyroidism diagnosed in this study, but the few tests carried out for the detection of these pollutants were negative. No direct relationship between the GB and the TG conditions has been found, but it is assumed that the central role of the TH in energy metabolism can indirectly alter the condition of the GB. More studies are necessary to understand the role of the GB in the metabolism.

6 SUMMARY / RESUMEN / ZUSAMMENFASSUNG

6. 1 ENGLISH SUMMARY

The glycogen body (GB) is a specific structure of the spinal cord of birds, localized between the third lumbar vertebrae and the first sacral vertebrae in the *fossa rhomboidea spinalis*. It is composed of astroglial cells specialized in storing large amounts of glycogen derivatives. The factors that control filling and the release of glycogen derivatives by the structure are still unknown. The function of the GB is uncertain, and different hypotheses have been proposed, such as energy storage for the nervous tissue and the cerebrospinal fluid, a role in myelin synthesis, a nonfunctional remnant from reptilian ancestors, etc.

The pattern of activity of the thyroid gland (TG) differs between altricial and precocial hatchlings, although its development is the same. The altricial neonates do not have the perihatch peak of thyroid hormones (TH) and thermoregulation response during the first week of life which is typical of precocial chicks.

The egg contains a low content of glucose, and gluconeogenesis in the liver of the embryo is the only possible pathway for obtaining glucose from lipids and proteins. Gluconeogenesis is biotin-dependant because one of its key enzymes, pyruvate carboxylase (PC), is biotin-dependant.

Between the 1st of January and the 31st of October of 2003, all dead chicks (up to one month) and embryos in the last period of incubation in the Loro Parque parrot collection were collected. The study is the first study of the GB in psittaciformes and also tried to find out the causes of empty / hypotrophic GBs reported in previous observations in the same collection. Complete necropsy, microbiological and histopathological studies (using PAS, Turnbull's blue and HE stains) were performed to diagnose the cause of death of all chicks. Finally, the database was

composed of 110 animals, mainly from the nursery. The clinical picture mainly described were stunted chicks with dry and pale skin, globose head and sudden deaths during the first week of life.

The GB was found inside the lumbosacral spinal cord between the nervous tissue and presented and inverted triangular shape in the transversal sections. It was mainly composed of one type of irregular, large and polygonal cell. The major part of the cells was occupied by stores of glycogen derivatives, which stained bright red – pink using the PAS stain. It was well vascularized and the central canal passed through it.

The GBs were only found to be normal in seven (6.4%) cases, the rest (93.6%) presented deficiencies in the glycogen filling of the astroglial cells. Problems of athyroidism and hypothyroidism were diagnosed in 86.1% of the birds in which the TG were studied. Immaturity of some organs, retarded yolk sac, unphysiological fatty livers, underdeveloped lungs and retarded extramedullary haematopoiesis were some of the other histopathological findings observed in these chicks.

It is suggested that possible factors interfering with gluconeogenesis during the embryonic period, such as eggs with a low biotin content laid by hens with subclinical biotin deficiencies, may be the cause of empty and hypotrophic GBs. Empting of the structure prior to death (by an unknown pathway) as an attempt to save the chicks' life is also suspected. A lack of colloid inside the follicles of the TG has also been noted. A direct relationship of the GB with the TG has not been observed, but more studies are necessary.

6. 2. RESUMEN EN CASTELLANO

El cuerpo glucogénico (CG) es una estructura específica de la columna vertebral de las aves, localizado entre la tercera vértebra lumbar y la primera vértebra sacra en la *fossa rhomboidea spinalis*. Esta constituido por células astrogliales especializadas en almacenar grandes cantidades de derivados glucogénicos. Los factores que controlan el vaciado y llenado con derivados glucogénicos de la estructura son aún desconocidos en la actualidad. La función del CG es incierta, y se han propuesto distintas hipótesis, como reservorio energético para el tejido nervioso y el fluido cerebroespinal, implicación en la síntesis de mielina, vestigio no funcional de los antecesores reptilianos, etc.

El patrón de actividad de las glándulas tiroideas (GT) difiere entre pichones altriciales y precociales, aunque su desarrollo sea el mismo. Los neonatos altriciales no tienen el pico de hormonas tiroideas (HT) alrededor de la eclosión ni la capacidad de termorregulación durante la primera semana de vida típica de las especies precociales.

Los huevos tiene un bajo contenido en glucosa, y la gluconeogénesis en el hígado del embrión es la única vía posible para obtener glucosa de los lípidos y proteínas. La gluconeogénesis es dependiente de biotina porque uno de sus enzimas, la piruvato–carboxilasa (PC), lo es.

Entre el 1 de Enero y el 31 de Octubre del 2003, se recogieron todos los pollitos (hasta un mes de edad) y embriones muertos en el último período de incubación de la colección de loros de Loro Parque. El estudio es el primer estudio del CG en psitaciformes y también pretende resolver las causas de CGs vacíos / hipotróficos descritos en observaciones anteriores en la misma colección. Se realizaron necropsias completas, y estudios microbiológicos e histopatológicos (utilizando tinciones PAS, Turnbull's blue y HE) para diagnosticar la causa de muerte de todos los pichones. Finalmente, la base de datos contaba con 110 animales, principalmente de la nursery. El cuadro clínico mayormente descrito fueron pichones con

problemas de crecimiento, piel pálida y seca, cabeza globoide y muertes súbitas durante la primera semana de vida.

El CG se describió dentro de la columna vertebral lumbosacra entre el tejido nervioso, y presentaba una forma triangular inversa en las secciones transversales. Mayoritariamente estaba constituido por un solo tipo de células irregulares, grandes y poligonales. Gran parte de la célula se encontraba ocupada por derivados glucogénicos almacenados, que se teñían en rojo – rosa brillante con la tinción PAS. Estaba bien vascularizado y el canal central pasaba a través de él.

Solo en siete casos (6,4%) se encontró un CG normal, y el resto (93,6%) presentaba deficiencias de contenido en las células astrogliales. Problemas de atiroidismo e hipotiroidismo se diagnosticaron en 86,1% de los pájaros en los que se estudiaron las GT. Otros resultados histopatológicos observados en estos pichones fueron órganos inmaduros, retrasos en la reabsorción del saco vitelino, hígados grasos no fisiológicos, pulmones subdesarrollados y hematopoyesis extramedular retardada.

Se sugiere que la interferencia de posibles factores en la gluconeogénesis durante el período embrionario, como huevos con bajo contenido en biotina puestos por hembras con deficiencias subclínicas de biotina, pueden ser la causa de CGs vacíos o hipotróficos. También se sospecha del vaciado de la estructura previamente a la muerte (mediante una vía desconocida) como mecanismo para salvar la vida del pichón. La carencia de coloide dentro de los folículos de las GT es otro de los hallazgos. No se ha podido observar una relación directa entre el CG y las GT, son necesarios más estudios para revelar dicha posible correlación.

6. 3. DEUTSCHE ZUSAMMENFASSUNG

Der Glycogenkörper (GK) ist eine spezifische Struktur im Rückenmark der Vögel. Er liegt zwischen dem 3. Lumbalwirbel und 1. Sakralwirbel in der Fossa rhomboidea spinalis. Der GK enthält Astrogliazellen, die sich auf die Speicherung großer Mengen von Glycogen spezialisiert haben.

Die Faktoren, die Füllung und Abgabe von Glykogen im GK kontrollieren, sind noch unbekamt. Die Funktion des GK wird noch diskutiert und verschiedene Hypothesen sind vorgeschlagen worden, wie Energiespeicherung für das Nervengewebe und die Zerebrospinalflüssigkeit, eine Rolle bei der Myelinsynthese oder als ein funktionsloses Überbleibsel der phylogenetischen Vorfahren der Voegel (Reptilien).

Die Art der Aktivität der Schilddrüse (SD) ist unterschiedlich bei Nesthockern und Nestflüchtern, obgleich die embryonale Entwicklung gleich verläuft. Den Eintagsküken der Nesthocker fehlt der hohe Schilddrüsenhormonspiegel kurz vor dem Schlupf, der für die Thermoregulation der Nestflüchterküken während der ersten Lebenswoche notwendig ist.

Das Ei enthält nur wenig Glucose. Die Gluconeogenese in der Leber des Embryos ist der einzige mögliche Weg zur Synthese von Glucose aus Lipiden und Proteinen. Die Gluconeogenese ist Biotin-abhängig, weil eines der Schlüsselenzyme, die Pyruvatkarboxylase, Biotin-abhängig ist.

Die Loro Parque Fundacion stellte zwischen dem 1. Januar und 31. Oktober 2003 alle toten Papageienküken (bis zum Alter von 1 Monat) und Embryonen, die während der letzten Bruttage abgestorben waren, für eine histopathologische Untersuchung zur Verfügung. Diese Untersuchung ist die erste, die den GK von Psittaciformes bearbeitet und ausserdem versucht hat, die Ursachen fuer leere oder hypotrophische GK zu finden, die bei frueheren Beobachtungen in derselben Kollektion auftraten.

Ausfuehrliche Sektionen, mikrobiologische und histopathologische Untersuchungen (mit PAS-, Turnbull's blue- und HE- Faerbung) wurden durchgefuehrt, um die Todesursache aller Nestlinge festzustellen. Von 110 Tieren, hauptsaechlich aus der Babystation, wurde eine Datenbasis erarbeitet. Unterentwickelte Nestlinge mit trockener, blasser Haut, rundlichem Kopf und ploetzlichem Tod beschreiben das klinische Bild waehrend der ersten Lebenswoche.

Der GK wurde innerhalb des lumbosakralen Rueckenmarkes zwischen dem Nervengewebe gefunden und hat die Form eines umgekehrten Dreieckes in Querschnitten. Der GK war ueberwiegend aus einem ungleichmaessig grossen, polygonalen Zelltyp zusammengesetzt. Ein grosser Teil der Zellen speicherte Glycogenderivate, die sich in der PAS-Faerbung leuchtend rot darstellten. Der GK war gut vaskularisiert und der Zentralkanal verlief durch ihn.

Nur bei sieben Nestlingen wurde der GK als "normal" beurteilt. Alle übrigen Fälle (93,6 %) zeigten Mängel bezüglich des Füllungszustandes der Astrogliazellen Athyreose bzw. Hypothyreose wurden in 86,1% der Nestlinge festgestellt, bei denen die SD untersucht wurde. Eine Unreife verschiedener Organe, wie unvollständig resorbierter Dottersack, Fehlen der physiologischen Fettleber, unterentwickelte Lungen und verlängerte extramedulläre Hämatopoese, sind weitere Beispiele für histopathologische Diagnosen bei diesen Nestlingen.

Es wird gefolgert, dass Faktoren, wie Bruteier mit niedrigem Biotingehalt bzw. Bruthennen mit subklinischem Biotinmangel, mit der Gluconeogenese interferieren und die Ursache von leeren oder hypotrophischen GK sein koennen. Die Entleerung des GK kurz vor dem Tod (auf unbekannte Weise) wird als Versuch des Nestlings gewertet, sein Leben zu retten. Der Mangel von Schilddrüsenkolloid in den Follikeln wird zusätzlich negativ bewertet. Eine direkte Beziehung zwischen GK und SD wurde nicht festgestellt, aber weitere Untersuchungen sind notwendig.

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Appendix

Table 6: Determined Weights of Examined Birds as Compared to the Average Weight of Birds of the same Age (that were maintained in the Baby Station) and Lung Development

ID No.	Species, subspecies	Age (days)	Code of origin	Source	Weight (g)	Average weight (g) according to age	Weight				Lung			
							Under normal	Normal	Above normal	Unknown	Underdeveloped	Normal development	Autolytic	Not examined
1	Amazona amazonica	0	LV 850	DIS	?	9,6				X		X		
2	Amazona amazonica	0	LV 850	DIS	?	9,6				X		X		
3	Amazona amazonica	18	LV 850	BS	24	128,4	X				X			
4	Amazona auropalliata	0	LV 844	DIS	?	14,6				X			X	
5	Amazona autumnalis salvini	0	LV 644	DIS	?	14,4				X		X		
6	Amazona barbadensis	0	LV 784	DIS	?	10,2				X		X		
7	Amazona ochrocephala nattereri	11	LV 876	BS	32	30,7		X				X		
8	Amazona oratrix tresmariae	3	LV 852	BS	10	15,9	X				X			
9	Amazona pretrei	0	LV 791	DIS	?	9,3				X			X	
10	Amazona rhodocorytha	0	LV 1017	DIS	?	13,7				X		X		
11	Amazona rhodocorytha	0	LV 576	DIS	?	13,7				X				X
12	Amazona ventralis	0	AM 372	DIS	?	10,2				X		X		
13	Amazona vinacea	10	LV 1012	BS	27	40,3	X					X		
14	Amazona xanthops	5	LV 820	BS	13	14,5		X				X		
15	Amazona xanthops	7	LV 820	BS	20	16			X			X		
16	Ara ararauna	0	LV587	DIS	?	20,1				X			X	
17	Ara ararauna	0	LV 605	DIS	23,7	20,1			X			X		
18	Ara ararauna	1	LV 605	BS	25	20,1			X			X		
19	Ara ararauna	0	LV 626	DIS	?	20,1				X			X	

Table 6 (continued): Determined Weights of Examined Birds as Compared to the Average Weight of Birds of the same Age (that were maintained in the Baby Station) and Lung Development

ID No.	Species, subspecies	Age (days)	Code of origin	Source	Weight (g)	Average weight (g) according to age	Weight				Lung			
							Under normal	Normal	Above normal	Unknown	Underdeveloped	Normal development	Autolytic	Not examined
20	Ara ararauna	0	LV 626	DIS	?	20,1				X		X		
21	Ara ararauna	0	LV 626	DIS	?	20,1				X		X		
22	Ara ararauna	2	LV 626	BS	17	21,1	X					X		
23	Ara ararauna	2	LV 626	BS	19,9	21,1		X				X		
24	Ara glaucogularis	2	LV 588	BS	12,4	20,2	X					X		
25	Ara glaucogularis	3	LV 592	BS	19	20,2		X				X		
26	Ara glaucogularis	0	LV 600	DIS	?	19,1				X		X		
27	Ara glaucogularis	3	LV 600	BS	23,2	20,2			X			X		
28	Ara glaucogularis	3	LV 600	BS	20,5	20,2		X				X		
29	Ara glaucogularis	10	LV 600	Nest	30	50,4	X					X		
30	Ara glaucogularis	0	LV 623	DIS	?	19,1				X		X		
31	Ara macao	0	LV 601	DIS	?	19,1				X			X	
32	Ara macao	0	LV 601	DIS	?	19,1				X		X		
33	Ara macao	2	LV 601	BS	22	20,7		X						X
34	Ara macao	0	LV 630	DIS	?	19,1				X				X
35	Ara macao	0	LV 648	DIS	?	19,1				X			X	
36	Ara macao	8	LV 648	BS	33	38,7	X				X			
37	Ara rubrogenys	3	LV 658	BS	12,9	17,3	X							X
38	Ara severus	3	LV199B	BS	10,7	14,4	X							X
39	Ara severus	17	LV 199B	BS	47	43,3		X				X		
40	Aratinga solstitialis	5	LV 170	Nest	8	9,7	X				X			

Table 6 (continued): Determined Weights of Examined Birds as Compared to the Average Weight of Birds of the same Age (that were maintained in the Baby Station) and Lung Development

ID No.	Species, subspecies	Age (days)	Code of origin	Source	Weight (g)	Average weight (g) according to age	Weight				Lung			
							Under normal	Normal	Above normal	Unknown	Underdeveloped	Normal development	Autolytic	Not examined
41	Aratinga solstitialis	7	LV 171	BS	6	11,3	X				X			
42	Aratinga solstitialis	11	LV 171	BS	3	21,8	X				X			
43	Aratinga solstitialis	8	LV 173	BS	7	12,8	X							X
44	Aratinga solstitialis	14	LV 173	BS	7	25,5	X				X			
45	Cacatua leadbeateri	4	LV 34	BS	13	14,1		X				X		
46	Cacatua pastinator	0	LV 39	DIS	?	13,1				X			X	
47	Cacatua sulphurea citrinocristata	0	LV 47	DIS	7	13,7	X					X		
48	Cyclopsitta diophthalma	4	LV 947	Nest	?	?				X		X		
49	Deroptyus accipitrinus fuscifrons	19	LV679	BS	49	46,3		X				X		
50	Diopsittaca nobilis cumanensis	5	AM 378	BS	9	12,5	X					X		
51	Diopsittaca nobilis cumanensis	5	AM 378	BS	11	12,5		X				X		
52	Diopsittaca nobilis cumanensis	0	LV 698	DIS	?	8,5				X			X	
53	Diopsittaca nobilis cumanensis	6	LV 698	BS	11	13,6	X					X		
54	Diopsittaca nobilis cumanensis	12	LV 698	BS	10	31,6	X				X			
55	Diopsittaca nobilis nobilis	3	AM 377	BS	6	7,1	X					X		
56	Diopsittaca nobilis nobilis	8	AM 377	BS	7	5,9			X			X		
57	Diopsittaca nobilis nobilis	4	LV 689	BS	8,9	7,9			X			X		
58	Diopsittaca nobilis nobilis	4	LV 689	BS	6,9	7,9	X					X		
59	Diopsittaca nobilis nobilis	25	LV 689	BS	47,3	84	X					X		
60	Eclectus roratus roratus	19	AS 124	BS	24	55,8	X				X			
61	Eclectus roratus solomonensis	11	LV 262	BS	35	32,3		X				X		

Table 6 (continued): Determined Weights of Examined Birds as Compared to the Average Weight of Birds of the same Age (that were maintained in the Baby Station) and Lung Development

ID No.	Species, subspecies	Age (days)	Code of origin	Source	Weight (g)	Average weight (g) according to age	Weight				Lung			
							Under normal	Normal	Above normal	Unknown	Underdeveloped	Normal development	Autolytic	Not examined
62	Eolophus roseicapilla	0	LV 474	DIS	?	9,2				X		X		
63	Eolophus roseicapilla	22	LV 474	BS	93	61			X			X		
64	Eos squamata riciniata	6	LV 57	BS	5	?	X					X		
65	Forpus passerinus	0	LV 1000	DIS	?	?				X				X
66	Loriculus vernalis	15	LV 991	Nest	14	?	X				X			
67	Loriculus vernalis	18	LV 991	Nest	23	?		X				X		
68	Neophema splendida	18	AU 2	Nest	9	?	X					X		
69	Nymphicus hollandicus	21	DF 1066	Nest	57	?	X				X			
70	Orthopsittaca manilata	0	AM 380	DIS	?	?				X		X		
71	Pionites leucogaster leucogaster	4	LV 706	BS	6	7,8	X					X		
72	Pionites leucogaster leucogaster	0	LV 715	DIS	?	6,8				X		X		
73	Pionites melanocephalus	9	AM 253	BS	11,7	14,8	X				X			
74	Pionus menstruus	3	LV 915	BS	5	8,6	X							X
75	Pionus tumultuosus	1	LV 703	BS	14	9,1			X			X		
76	Pionus tumultuosus	2	LV 703	BS	8	10,3	X					X		
77	Pionus tumultuosus	3	LV 703	BS	12	11,5		X				X		
78	Poicephalus robustus fuscicollis	18	LV 378	BS	43	65,3	X				X			
79	Poicephalus robustus fuscicollis	1	LV 383	BS	9	9,7		X				X		
80	Poicephalus rufiventris	8	AF5	BS	7	11,1	X							X
81	Poicephalus rufiventris	8	LV 376	BS	10	11,1		X						X
82	Poicephalus rufiventris	8	LV 376	BS	12	11,1		X				X		

Table 6 (continued): Determined Weights of Examined Birds as Compared to the Average Weight of Birds of the same Age (that were maintained in the Baby Station) and Lung Development

ID No.	Species, subspecies	Age (days)	Code of origin	Source	Weight (g)	Average weight (g) according to age	Weight				Lung			
							Under normal	Normal	Above normal	Unknown	Underdeveloped	Normal development	Autolytic	Not examined
83	Poicephalus rufiventris	15	LV 376	BS	26	21,6			X			X		
84	Poicephalus rufiventris	19	LV 376	BS	18	32,4	X				X			
85	Poicephalus rufiventris	0	LV 380	DIS	?	5,8				X		X		
86	Poicephalus rufiventris	12	LV380	BS	19	16,1			X		X			
87	Primolius auricollis	2	AM 382	BS	14	12,5			X			X		
88	Primolius couloni	2	AM 379	BS	10	12,8	X					X		
89	Primolius couloni	15	LV 671	BS	96	57,8			X			X		
90	Primolius couloni	17	LV 671	BS	159	74,1			X		X			
91	Primolius couloni	21	LV 671	BS	306	92,4			X			X		
92	Primolius maracana	0	LV 419	DIS	?	11,3				X		X		
93	Primolius maracana	0	LV 419	DIS	10,6	11,3		X				X		
94	Primolius maracana	15	LV 419	BS	15,6	63,5	X				X			
95	Primolius maracana	3	LV 423	BS	11	13,4	X					X		
96	Psittacula alexandri abbotti	2	LV 236	Nest	?	5,4				X		X		
97	Psittacula cyanocephala	10	LV 218	BS	5	7,9	X				X			
98	Psittacula cyanocephala	18	LV 218	BS	6	16,4	X				X			
99	Psittacula krameri manillensis	6	LV 217	Nest	4	?	X							X
100	Psittacus erithacus timneh	11	LV 514	BS	18	30	X				X			
101	Pyrrhura lepida	0	LV 284	DIS	?	?				X				X
102	Pyrrhura perlata	2	LV 145	BS	3,2	?		X				X		
103	Rhynchopsitta pachyrhyncha	3	LV678	BS	11,3	13,7	X					X		

Table 6 (continued): Determined Weights of Examined Birds as Compared to the Average Weight of Birds of the same Age (that were maintained in the Baby Station) and Lung Development

ID No.	Species, subspecies	Age (days)	Code of origin	Source	Weight (g)	Average weight (g) according to age	Weight				Lung			
							Under normal	Normal	Above normal	Unknown	Underdeveloped	Normal development	Autolytic	Not examined
104	Trichoglossus capistratus	4	LV 98	BS	8	6,4			X			X		
105	Trichoglossus haematodus caeruleiceps	0	LV 49	DIS	?	4,6				X		X		
106	Trichoglossus haematodus caeruleiceps	9	LV49	BS	12	9,5			X		X			
107	Trichoglossus haematodus caeruleiceps	10	LV 49	BS	6,3	10,3	X					X		
108	Trichoglossus rubritorquis	35	LV 84	BS	23	107	X					X		
109	Triclaria malachitacea	3	LV 735	Nest	?	11,1				X		X		
110	Triclaria malachitacea (egg twins)	0	LV 735	DIS	?	6,4				X			X	
						Total	43	18	16	33	21	68	9	12

?: Unknown weight

Average weight according to age: Average calculated from the daily weights database of the Baby station in Loro Parque of 4 breeding seasons

Under normal weight: Birds weighing less than the normal weight

Normal weight: +/- 10% the average weight

Above weight: Birds weighing more than the normal weight

Table 8. Glycogen Body and Thyroid Gland Status

ID No.	Species, subspecies	Age (days)	Code of origin	Empty GB	Sev. hypotrophic GB	Mod. hypotrophic GB	Normal GB	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid not examined
1	Amazona amazonica	0	LV 850	X							X
2	Amazona amazonica	0	LV 850		X			X			
3	Amazona amazonica	18	LV 850		X						X
4	Amazona auropalliata	0	LV 844		X			X			
5	Amazona autumnalis salvini	0	LV 644	X				X			
6	Amazona barbadensis	0	LV 784	X							X
7	Amazona ochrocephala nattereri	11	LV 876				X			X	
8	Amazona oratrix tresmariae	3	LV 852		X			X			
9	Amazona pretrei	0	LV 791	X				X			
10	Amazona rhodocorytha	0	LV 1017		X				X		
11	Amazona rhodocorytha	0	LV 576	X				X			
12	Amazona ventralis	0	AM 372		X			X			
13	Amazona vinacea	10	LV 1012		X					X	
14	Amazona xanthops	5	LV 820		X				X		
15	Amazona xanthops	7	LV 820		X			X			
16	Ara ararauna	0	LV 587		X						X
17	Ara ararauna	0	LV 605				X	X			
18	Ara ararauna	1	LV 605		X			X			
19	Ara ararauna	0	LV 626	X				X			
20	Ara ararauna	0	LV 626			X					X
21	Ara ararauna	0	LV 626	X							X
22	Ara ararauna	2	LV 626		X			X			
23	Ara ararauna	2	LV 626			X		X			
24	Ara glaucogularis	2	LV 588	X				X			
25	Ara glaucogularis	3	LV 592		X			X			
26	Ara glaucogularis	0	LV 600	X				X			
27	Ara glaucogularis	3	LV 600		X			X			
28	Ara glaucogularis	3	LV 600		X					X	
29	Ara glaucogularis	10	LV 600	X				X			
30	Ara glaucogularis	0	LV 623	X				X			
31	Ara macao	0	LV 601				X				X
32	Ara macao	0	LV 601			X		X			
33	Ara macao	2	LV 601			X			X		

Table 8 (continued). Glycogen Body and Thyroid Gland Status

ID No.	Species, subspecies	Age (days)	Code of origin	Empty GB	Sev. hypotrophic GB	Mod. hypotrophic GB	Normal GB	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid not examined
34	Ara macao	0	LV 630	X				X			
35	Ara macao	0	LV 648	X				X			
36	Ara macao	8	LV 648				X		X		
37	Ara rubrogenys	3	LV 658		X			X			
38	Ara severus	3	LV 199B		X			X			
39	Ara severus	17	LV 199B			X		X			
40	Aratinga solstitialis	5	LV 170		X			X			
41	Aratinga solstitialis	7	LV 171		X						X
42	Aratinga solstitialis	11	LV 171	X							X
43	Aratinga solstitialis	8	LV 173	X							X
44	Aratinga solstitialis	14	LV 173		X			X			
45	Cacatua leadbeateri	4	LV 34	X							X
46	Cacatua pastinator	0	LV 39	X							X
47	Cacatua sulphurea citrinocristata	0	LV 47		X			X			
48	Cyclopsitta diophthalma	4	LV 947	X				X			
49	Deroptyus accipitrinus fuscifrons	19	LV 679		X					X	
50	Diopsittaca nobilis cumanensis	5	AM 378		X					X	
51	Diopsittaca nobilis cumanensis	5	AM 378		X						X
52	Diopsittaca nobilis cumanensis	0	LV 698		X						X
53	Diopsittaca nobilis cumanensis	6	LV 698		X						X
54	Diopsittaca nobilis cumanensis	12	LV 698				X		X		
55	Diopsittaca nobilis nobilis	3	AM 377			X		X			
56	Diopsittaca nobilis nobilis	8	AM 377		X					X	
57	Diopsittaca nobilis nobilis	4	LV 689		X						X
58	Diopsittaca nobilis nobilis	4	LV 689		X			X			
59	Diopsittaca nobilis nobilis	25	LV 689			X			X		
60	Eclectus roratus roratus	19	AS 124	X							X
61	Eclectus roratus solomonensis	11	LV 262		X			X			
62	Eolophus roseicapilla	0	LV 474	X				X			
63	Eolophus roseicapilla	22	LV 474	X					X		
64	Eos squamata riciniata	6	LV 57	X							X
65	Forpus passerinus	0	LV 1000	X							X
66	Loriculus vernalis	15	LV 991		X			X			
67	Loriculus vernalis	18	LV 991		X			X			

Table 8 (continued). Glycogen Body and Thyroid Gland Status

ID No.	Species, subspecies	Age (days)	Code of origin	Empty GB	Sev. hypotrophic GB	Mod. hypotrophic GB	Normal GB	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid not examined
68	Neophema splendida	18	AU 2	X				X			
69	Nymphicus hollandicus	21	DF 1066	X						X	
70	Orthopsittaca manilata	0	AM 380		X			X			
71	Pionites leucogaster leucogaster	4	LV 706		X						X
72	Pionites leucogaster leucogaster	0	LV 715	X				X			
73	Pionites melanocephalus	9	AM 253		X				X		
74	Pionus menstruus	3	LV 915		X			X			
75	Pionus tumultuosus	1	LV 703	X				X			
76	Pionus tumultuosus	2	LV 703		X			X			
77	Pionus tumultuosus	3	LV 703		X			X			
78	Poicephalus robustus fuscicollis	18	LV 378	X						X	
79	Poicephalus robustus fuscicollis	1	LV 383		X						X
80	Poicephalus rufiventris	8	AF 5		X				X		
81	Poicephalus rufiventris	8	LV 376	X							X
82	Poicephalus rufiventris	8	LV 376		X					X	
83	Poicephalus rufiventris	15	LV 376		X				X		
84	Poicephalus rufiventris	19	LV 376			X			X		
85	Poicephalus rufiventris	0	LV 380			X					X
86	Poicephalus rufiventris	12	LV 380				X				X
87	Primolius auricollis	2	AM 382			X		X			
88	Primolius couloni	2	AM 379		X			X			
89	Primolius couloni	15	LV 671	X				X			
90	Primolius couloni	17	LV 671		X						X
91	Primolius couloni	21	LV 671		X			X			
92	Primolius maracana	0	LV 419	X				X			
93	Primolius maracana	0	LV 419		X			X			
94	Primolius maracana	15	LV 419	X				X			
95	Primolius maracana	3	LV 423		X			X			
96	Psittacula alexandri abbotti	2	LV 236		X						X
97	Psittacula cyanocephala	10	LV 218	X				X			
98	Psittacula cyanocephala	18	LV 218		X					X	
99	Psittacula krameri manillensis	6	LV 217		X					X	
100	Psittacus erithacus timneh	11	LV 514				X	X			

Table 8 (continued). Glycogen Body and Thyroid Gland Status

ID No.	Species, subspecies	Age (days)	Code of origin	Empty GB	Sev. hypotrophic GB	Mod. hypotrophic GB	Normal GB	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid not examined
101	Pyrrhura lepida	0	LV 284		X						X
102	Pyrrhura perlata	2	LV 145		X				X		
103	Rhynchopsitta pachyrhyncha	3	LV 678		X			X			
104	Trichoglossus capistratus	4	LV 98	X				X			
105	Trichoglossus haematodus caeruleiceps	0	LV 49	X							X
106	Trichoglossus haematodus caeruleiceps	9	LV 49			X					X
107	Trichoglossus haematodus caeruleiceps	10	LV 49		X				X		
108	Trichoglossus rubritorquis	35	LV 84		X			X			
109	Triclaria malachitacea	3	LV 735	X							X
110	Triclaria malachitacea (egg twins)	0	LV 735	X							X
			Total	**37**	**55**	**11**	**7**	**55**	**13**	**11**	**31**

Empty GB: following PAS-stain, no glycogen detectable filling the cells
Severely hypotrophic GB: following PAS-stain, only remnants of glycogen detectable filling the cells
Moderately hypotrophic GB: following PAS-stain, estimate 2/3 of the area filled with glycogen
Normal GB: following PAS-stain, GB cells completely filled with glycogen

X Slight hypothyroidism in the first week considered physiological (quiescent thryroid)
Athyroidism: following PAS and H-E stain, collapsed or empty follicles with cuboidal - cylindrical epithelium
Hypothyroidism: following PAS and H-E stain, follicles partially empty or being emptied and cuboidal - cylindrical epithelium
Quiescent thryoid: following PAS and H-E stain, follicles entirely filled with colloid and rather flat epithelium Normal thyroid
Thyroid not examined: The thyroid could not be studied

Table 12: Yolk Sac and Fatty Liver Status. Evaluation of the Fat Stores Use

ID No.	Species, subspecies	Age (days)	Code of origin	Yolk sac					Liver										
				Empty	Remnants	Moderately filled	Full	Broken	No fatty liver	Slight fatty liver	Moderate fatty liver	Heavy fatty liver	Autolytic	Not examined	Yolk in the intestines	Early use of the yolk sac	Retarded yolk sac	No proper fatty liver	Proper yolk/ phys.fat.liver use
1	Amazona amazonica	0	LV 850			X							X		X	X			
2	Amazona amazonica	0	LV 850				X						X		X	X			
3	Amazona amazonica	18	LV 850	X					X										X
4	Amazona auropalliata	0	LV 844			X				X					X	X			
5	Amazona autumnalis salvini	0	LV 644					X					X		X	X			
6	Amazona barbadensis	0	LV 784					X					X		X	X			
7	Amazona ochrocephala nattereri	11	LV 876		X						X						X	X	
8	Amazona oratrix tresmariae	3	LV 852			X			X									X	
9	Amazona pretrei	0	LV 791		X								X		X	X			
10	Amazona rhodocorytha	0	LV 1017				X			X									X
11	Amazona rhodocorytha	0	LV 576			X				X					X	X			
12	Amazona ventralis	0	AM 372			X							X		X	X			
13	Amazona vinacea	10	LV 1012	X					X										X
14	Amazona xanthops	5	LV 820	X					X							X		X	
15	Amazona xanthops	7	LV 820		X						X								X
16	Ara ararauna	0	LV 587		X								X		X	X			
17	Ara ararauna	0	LV 605				X			X									X
18	Ara ararauna	1	LV 605				X			X									X
19	Ara ararauna	0	LV 626		X					X					X	X			
20	Ara ararauna	0	LV 626		X					X					X	X			
21	Ara ararauna	0	LV 626					X	X						X	X			

Table 12 (continued): Yolk Sac and Fatty Liver Status. Evaluation of the Fat Stores Use

ID No.	Species, subspecies	Age (days)	Code of origin	Yolk sac					Liver										
				Empty	Remnants	Moderately filled	Full	Broken	No fatty liver	Slight fatty liver	Moderate fatty liver	Heavy fatty liver	Autolytic	Not examined	Yolk in the intestines	Early use of the yolk sac	Retarded yolk sac	No proper fatty liver	Proper yolk/ phys.fat.liver use
22	Ara ararauna	2	LV 626				X			X									X
23	Ara ararauna	2	LV 626			X					X								X
24	Ara glaucogularis	2	LV 588			X					X								X
25	Ara glaucogularis	3	LV 592	X						X						X		X	
26	Ara glaucogularis	0	LV 600		X						X				X	X			
27	Ara glaucogularis	3	LV 600			X				X								X	
28	Ara glaucogularis	3	LV 600			X			X									X	
29	Ara glaucogularis	10	LV 600		X					X							X		
30	Ara glaucogularis	0	LV 623		X								X		X	X			
31	Ara macao	0	LV 601			X							X		X	X			
32	Ara macao	0	LV 601			X							X		X	X			
33	Ara macao	2	LV 601			X					X								X
34	Ara macao	0	LV 630			X				X					X	X			
35	Ara macao	0	LV 648		X								X		X	X			
36	Ara macao	8	LV 648	X						X									X
37	Ara rubrogenys	3	LV 658			X			X								X	X	
38	Ara severus	3	LV 199B		X						X								X
39	Ara severus	17	LV 199B		X					X							X	X	
40	Aratinga solstitialis	5	LV 170		X				X									X	
41	Aratinga solstitialis	7	LV 171	X						X									X
42	Aratinga solstitialis	11	LV 171		X				X								X		

Table 12 (continued): Yolk Sac and Fatty Liver Status. Evaluation of the Fat Stores Use

ID No.	Species, subspecies	Age (days)	Code of origin	Yolk sac					Liver										
				Empty	Remnants	Moderately filled	Full	Broken	No fatty liver	Slight fatty liver	Moderate fatty liver	Heavy fatty liver	Autolytic	Not examined	Yolk in the intestines	Early use of the yolk sac	Retarded yolk sac	No proper fatty liver	Proper yolk/ phys.fat.liver use
43	Aratinga solstitialis	8	LV 173	X						X									X
44	Aratinga solstitialis	14	LV 173	X						X								X	
45	Cacatua leadbeateri	4	LV 34	X						X						X		X	
46	Cacatua pastinator	0	LV 39		X								X		X	X			
47	Cacatua sulphurea citrinocristata	0	LV 47		X					X					X	X			
48	Cyclopsitta diophthalma	4	LV 947	X					X							X		X	
49	Deroptyus accipitrinus fuscifrons	19	LV 679	X					X										X
50	Diopsittaca nobilis cumanensis	5	AM 378	X						X								X	
51	Diopsittaca nobilis cumanensis	5	AM 378	X							X								X
52	Diopsittaca nobilis cumanensis	0	LV 698			X							X		X	X			
53	Diopsittaca nobilis cumanensis	6	LV 698	X						X									X
54	Diopsittaca nobilis cumanensis	12	LV 698	X							X							X	
55	Diopsittaca nobilis nobilis	3	AM 377	X					X							X		X	
56	Diopsittaca nobilis nobilis	8	AM 377	X							X							X	
57	Diopsittaca nobilis nobilis	4	LV 689	X								X							X
58	Diopsittaca nobilis nobilis	4	LV 689		X				X									X	
59	Diopsittaca nobilis nobilis	25	LV 689	X							X							X	
60	Eclectus roratus roratus	19	AS 124	X						X								X	
61	Eclectus roratus solomonensis	11	LV 262		X						X						X	X	
62	Eolophus roseicapilla	0	LV 474			X								X	X	X			
63	Eolophus roseicapilla	22	LV 474	X						X								X	

Table 12 (continued): Yolk Sac and Fatty Liver Status. Evaluation of the Fat Stores Use

ID No.	Species, subspecies	Age (days)	Code of origin	Yolk sac					Liver						Yolk in the intestines	Early use of the yolk sac	Retarded yolk sac	No proper fatty liver	Proper yolk/ phys.fat.liver use
				Empty	Remnants	Moderately filled	Full	Broken	No fatty liver	Slight fatty liver	Moderate fatty liver	Heavy fatty liver	Autolytic	Not examined					
64	Eos squamata riciniata	6	LV 57		X						X								X
65	Forpus passerinus	0	LV 1000			X							X		X	X			
66	Loriculus vernalis	15	LV 991	X						X								X	
67	Loriculus vernalis	18	LV 991	X							X							X	
68	Neophema splendida	18	AU 2	X					X										X
69	Nymphicus hollandicus	21	DF 1066	X					X										X
70	Orthopsittaca manilata	0	AM 380		X								X		X	X			
71	Pionites leucogaster leucogaster	4	LV 706	X							X								X
72	Pionites leucogaster leucogaster	0	LV 715	X						X						X			
73	Pionites melanocephalus	9	AM 253	X					X										X
74	Pionus menstruus	3	LV 915					X	X									X	
75	Pionus tumultuosus	1	LV 703		X					X						X			
76	Pionus tumultuosus	2	LV 703		X				X							X		X	
77	Pionus tumultuosus	3	LV 703		X						X								X
78	Poicephalus robustus fuscicollis	18	LV 378			X			X								X		
79	Poicephalus robustus fuscicollis	1	LV 383			X					X								X
80	Poicephalus rufiventris	8	AF 5	X										X					X
81	Poicephalus rufiventris	8	LV 376	X						X									X
82	Poicephalus rufiventris	8	LV 376	X						X									X
83	Poicephalus rufiventris	15	LV 376	X							X							X	
84	Poicephalus rufiventris	19	LV 376	X							X							X	

Table 12 (continued): Yolk Sac and Fatty Liver Status. Evaluation of the Fat Stores Use

ID No.	Species, subspecies	Age (days)	Code of origin	Yolk sac					Liver						Yolk in the intestines	Early use of the yolk sac	Retarded yolk sac	No proper fatty liver	Proper yolk/phys.fat.liver use
				Empty	Remnants	Moderately filled	Full	Broken	No fatty liver	Slight fatty liver	Moderate fatty liver	Heavy fatty liver	Autolytic	Not examined					
85	Poicephalus rufiventris	0	LV 380		X								X			X			
86	Poicephalus rufiventris	12	LV 380	X						X								X	
87	Primolius auricollis	2	AM 382			X					X								X
88	Primolius couloni	2	AM 379	X						X						X			
89	Primolius couloni	15	LV 671	X							X							X	
90	Primolius couloni	17	LV 671	X						X								X	
91	Primolius couloni	21	LV 671	X								X						X	
92	Primolius maracana	0	LV 419					X	X										X
93	Primolius maracana	0	LV 419		X					X						X			
94	Primolius maracana	15	LV 419			X			X								X		
95	Primolius maracana	3	LV 423		X				X									X	
96	Psittacula alexandri abbotti	2	LV 236					X	X									X	
97	Psittacula cyanocephala	10	LV 218		X				X								X		
98	Psittacula cyanocephala	18	LV 218	X							X							X	
99	Psittacula krameri manillensis	6	LV 217	X					X									X	
100	Psittacus erithacus timneh	11	LV 514	X							X							X	
101	Pyrrhura lepida	0	LV 284					X						X					
102	Pyrrhura perlata	2	LV 145			X			X									X	
103	Rhynchopsitta pachyrhyncha	3	LV 678				X			X							X	X	
104	Trichoglossus capistratus	4	LV 98	X						X								X	

Table 12 (continued): Yolk Sac and Fatty Liver Status. Evaluation of the Fat Stores Use

ID No.	Species, subspecies	Age (days)	Code of origin	Yolk sac					Liver										
				Empty	Remnants	Moderately filled	Full	Broken	No fatty liver	Slight fatty liver	Moderate fatty liver	Heavy fatty liver	Autolytic	Not examined	Yolk in the intestines	Early use of the yolk sac	Retarded yolk sac	No proper fatty liver	Proper yolk/phys.fat.liver use
105	Trichoglossus haematodus caeruleiceps	0	LV 49		X								X		X	X			
106	Trichoglossus haematodus caeruleiceps	9	LV 49	X						X									X
107	Trichoglossus haematodus caeruleiceps	10	LV 49		X				X								X		
108	Trichoglossus rubritorquis	35	LV 84	X					X										X
109	Triclaria malachitacea	3	LV 735	X						X								X	
110	Triclaria malachitacea (egg twins)	0	LV 735			X							X		X	X			
			Total	**45**	**29**	**23**	**6**	**7**	**28**	**36**	**23**	**2**	**18**	**3**	**26**	**37**	**11**	**40**	**31**

Empty yolk sac: following PAS stain, no yolk (PAS + material) inside the sac
Remnants in the yolk sac: following PAS stain, just remnants of yolk (PAS + material) inside the sac
Moderately filled yolk sac: following PAS stain, estimated 2/3 - 1/2 of the sac full of yolk (PAS + material)
Full yolk sac: following PAS stain, sac full or almost full of yolk (PAS + material)
Broken yolk sac: Yolk sac accidentally broken during the necropsy
No fatty liver: following H-E stain, no presence of fat vacuoles inside the hepatocytes
Slight fatty liver: following H-E stain, few fat vacuoles inside the hepatocytes or few hepatocytes with fat storage
Moderate fatty liver: following H-E stain, estimated 1/3 - 1/2 of the hepatocytes with fat storage
Heavy fatty liver: following H-E stain, estimated 2/3 of the hepatocytes with fat vacuoles
Autolytic liver: following H-E stain, erythrocytes with broken nuclei, brownish discoloration of the cytoplasma and damaged hepatocytes (broken nuclei/ membranes)
Not examined fatty liver: liver not cut in the histological processing
Yolk in the intestines: following PAS stain, presence of yolk (PAS + material) in the intestinal lumen
Early use of the yolk sac: presence of yolk (PAS + material) inside the intestines before hatching, or low fat stores (yolk sac and fatty liver) according to the age
Retarded yolk sac: large amounts or still remnants of yolk (PAS + material) inside the sac, which probably has not been used properly, according to the age
No proper fatty liver: Poor amount of fat stored in the hepatocytes according to the age
X Retarded physiologically fattty liver

Table 13: Intestine Findings

ID No.	Species, subspecies	Age (days)	Code of origin	Intestines		
				Oedema in the villi	Immature	Other findings
17	Ara ararauna	0	LV 605	X		
23	Ara ararauna	2	LV 626	X		
25	Ara glaucogularis	3	LV 592	X		
26	Ara glaucogularis	0	LV 600		X	
27	Ara glaucogularis	3	LV 600			Intussusception
29	Ara glaucogularis	10	LV 600			Few lymphoid follicles (early)
30	Ara glaucogularis	0	LV 623	X		
33	Ara macao	2	LV 601	X		
37	Ara rubrogenys	3	LV 658	X		
38	Ara severus	3	LV 199B	X		
66	Loriculus vernalis	15	LV 991	X		
73	Pionites melanocephalus	9	AM 253	X		
75	Pionus tumultuosus	1	LV 703	X		
77	Pionus tumultuosus	3	LV 703		X	
87	Primolius auricollis	2	AM 382	X		
88	Primolius couloni	2	AM 379		X	
91	Primolius couloni	21	LV 671			Severe hyperaemia
95	Primolius maracana	3	LV 423	X	X	
98	Psittacula cyanocephala	18	LV 218	X		
99	Psittacula krameri manillensis	6	LV 217	X		
103	Rhynchopsitta pachyrhyncha	3	LV 678	X		
106	Trichoglossus haematodus caeruleiceps	9	LV 49	X		
107	Trichoglossus haematodus caeruleiceps	10	LV 49		X	Iron block positive
			Total	16	5	

In the birds not listed, the intestines were either autolytic, not examined, or did not show obvious lesions

Immature intestines: Extremely short villi, specially in the duodenum

Table 14: Pancreas Findings

ID No.	Species, subspecies	Age (days)	Code of origin	Pancreas			
				No or few zymogenic granules	Degeneration of exocrinic pancreas	Degeneration of endocrinic pancreas	Other findings
14	Amazona xanthops	5	LV 820	X	X		
15	Amazona xanthops	7	LV 820	X			Oedema exocrinic pancreas
33	Ara macao	2	LV 601	X			
36	Ara macao	8	LV 648			X	Diagnosis: Diabetes mellitus
37	Ara rubrogenys	3	LV 658	X			
38	Ara severus	3	LV 199B	X			Oedema exocrinic pancreas
48	Cyclopsitta diophthalma	4	LV 947	X			
49	Deroptyus accipitrinus fuscifrons	19	LV 679	X			
50	Diopsittaca nobilis cumanensis	5	AM 378	X			
51	Diopsittaca nobilis cumanensis	5	AM 378			X	Diagnosis: Diabetes mellitus
53	Diopsittaca nobilis cumanensis	6	LV 698	X			
54	Diopsittaca nobilis cumanensis	12	LV 698	X	X		
56	Diopsittaca nobilis nobilis	8	AM 377	X	X		
59	Diopsittaca nobilis nobilis	25	LV 689	X			
73	Pionites melanocephalus	9	AM 253	X			
74	Pionus menstruus	3	LV 915	X			
83	Poicephalus rufiventris	15	LV 376	X			
103	Rhynchopsitta pachyrhyncha	3	LV 678	X			Oedema exocrinic pancreas
			Total	**16**	**3**	**2**	

In the birds not listed, the pancreas was either autolytic, not examined, or did not show obvious lesions

Degeneration of exocrinic pancreas: Vacuolation, atrophy or hypotrophy of exocrinic cells

Degeneration of endocrinic pancreas: Vacuolation, atrophy or hypotrophy of islet cells

Table 15: Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver													
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
1	Amazona amazonica	0	LV 850													X	
2	Amazona amazonica	0	LV 850													X	
3	Amazona amazonica	18	LV 850											X	X		
4	Amazona auropalliata	0	LV 844													X	
5	Amazona autumnalis salvini	0	LV 644													X	
6	Amazona barbadensis	0	LV 784													X	
7	Amazona ochrocephala nattereri	11	LV 876		X					Hepatocytes without border lines							
8	Amazona oratrix tresmariae	3	LV 852	X													
9	Amazona pretrei	0	LV 791													X	
10	Amazona rhodocorytha	0	LV 1017												X		
11	Amazona rhodocorytha	0	LV 576													X	
12	Amazona ventralis	0	AM 372													X	
13	Amazona vinacea	10	LV 1012					X									
14	Amazona xanthops	5	LV 820	X													
15	Amazona xanthops	7	LV 820												X		

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver												
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
16	Ara ararauna	0	LV 587													X	
17	Ara ararauna	0	LV 605												X		
18	Ara ararauna	1	LV 605						X								
19	Ara ararauna	0	LV 626												X		
20	Ara ararauna	0	LV 626												X		
21	Ara ararauna	0	LV 626												X		
22	Ara ararauna	2	LV 626												X		
23	Ara ararauna	2	LV 626												X		
24	Ara glaucogularis	2	LV 588												X		
25	Ara glaucogularis	3	LV 592	X													
26	Ara glaucogularis	0	LV 600													X	
27	Ara glaucogularis	3	LV 600	X													
28	Ara glaucogularis	3	LV 600	X													
29	Ara glaucogularis	10	LV 600												X		
30	Ara glaucogularis	0	LV 623													X	

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver														
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
31	Ara macao	0	LV 601													X	
32	Ara macao	0	LV 601													X	
33	Ara macao	2	LV 601												X		
34	Ara macao	0	LV 630													X	
35	Ara macao	0	LV 648													X	
36	Ara macao	8	LV 648										X		X		
37	Ara rubrogenys	3	LV 658	X									X				
38	Ara severus	3	LV 199B												X		
39	Ara severus	17	LV 199B		X			X						X			
40	Aratinga solstitialis	5	LV 170	X													
41	Aratinga solstitialis	7	LV 171												X		
42	Aratinga solstitialis	11	LV 171						X					X			
43	Aratinga solstitialis	8	LV 173											X	X		
44	Aratinga solstitialis	14	LV 173		X									X			
45	Cacatua leadbeateri	4	LV 34	X													

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver														
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
46	Cacatua pastinator	0	LV 39													X	
47	Cacatua sulphurea citrinocristata	0	LV 47												X		
48	Cyclopsitta diophthalma	4	LV 947	X													
49	Deroptyus accipitrinus fuscifrons	19	LV 679							X				X			
50	Diopsittaca nobilis cumanensis	5	AM 378	X								X					
51	Diopsittaca nobilis cumanensis	5	AM 378												X		
52	Diopsittaca nobilis cumanensis	0	LV 698													X	
53	Diopsittaca nobilis cumanensis	6	LV 698			X											
54	Diopsittaca nobilis cumanensis	12	LV 698		X							X					
55	Diopsittaca nobilis nobilis	3	AM 377	X													
56	Diopsittaca nobilis nobilis	8	AM 377		X												
57	Diopsittaca nobilis nobilis	4	LV 689									Glycogen storage disease					
58	Diopsittaca nobilis nobilis	4	LV 689	X													
59	Diopsittaca nobilis nobilis	25	LV 689		X	X	X							X			
60	Eclectus roratus roratus	19	AS 124		X				X					X			

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver													
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
61	Eclectus roratus solomonensis	11	LV 262		X												
62	Eolophus roseicapilla	0	LV 474														X
63	Eolophus roseicapilla	22	LV 474		X			X	X								
64	Eos squamata riciniata	6	LV 57												X		
65	Forpus passerinus	0	LV 1000													X	
66	Loriculus vernalis	15	LV 991		X									X			
67	Loriculus vernalis	18	LV 991		X	X								X			
68	Neophema splendida	18	AU 2					X				X					
69	Nymphicus hollandicus	21	DF 1066			X											
70	Orthopsittaca manilata	0	AM 380													X	
71	Pionites leucogaster leucogaster	4	LV 706			X							X				
72	Pionites leucogaster leucogaster	0	LV 715													X	
73	Pionites melanocephalus	9	AM 253											X	X		
74	Pionus menstruus	3	LV 915	X													
75	Pionus tumultuosus	1	LV 703										X		X		

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver													
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
76	Pionus tumultuosus	2	LV 703	X													
77	Pionus tumultuosus	3	LV 703			X						X	X				
78	Poicephalus robustus fuscicollis	18	LV 378											X	X		
79	Poicephalus robustus fuscicollis	1	LV 383												X		
80	Poicephalus rufiventris	8	AF 5														X
81	Poicephalus rufiventris	8	LV 376								X						
82	Poicephalus rufiventris	8	LV 376					X	X		X						
83	Poicephalus rufiventris	15	LV 376		X									X			
84	Poicephalus rufiventris	19	LV 376		X	X								X			
85	Poicephalus rufiventris	0	LV 380													X	
86	Poicephalus rufiventris	12	LV 380		X									X			
87	Primolius auricollis	2	AM 382									Glycogen storage disease					
88	Primolius couloni	2	AM 379					X	X								
89	Primolius couloni	15	LV 671		X	X								X			
90	Primolius couloni	17	LV 671		X	X								X			

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver													
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
91	Primolius couloni	21	LV 671		X	X								X			
92	Primolius maracana	0	LV 419												X		
93	Primolius maracana	0	LV 419												X		
94	Primolius maracana	15	LV 419						X								
95	Primolius maracana	3	LV 423	X													
96	Psittacula alexandri abbotti	2	LV 236	X													
97	Psittacula cyanocephala	10	LV 218							X				X			
98	Psittacula cyanocephala	18	LV 218		X												
99	Psittacula krameri manillensis	6	LV 217	X					X								
100	Psittacus erithacus timneh	11	LV 514		X					X				X			
101	Pyrrhura lepida	0	LV 284														X
102	Pyrrhura perlata	2	LV 145	X													
103	Rhynchopsitta pachyrhyncha	3	LV 678	X					X								
104	Trichoglossus capistratus	4	LV 98	X													
105	Trichoglossus haematodus caeruleiceps	0	LV 49													X	

Table 15 (continued): Histological Evaluation of the Liver

ID No.	Species, subspecies	Age (days)	Code of origin	Liver													
				No proper fatty liver	Retarded fatty liver	Fatty liver degeneration	Hepatitis	Hepatosis	Necrosis	Haemosiderosis	Haemochromatosis	Anaemia	Poor extramed. haematopoiesis	Retarded extramed. haematopoiesis	Normal	Autolytic	Not examined
106	Trichoglossus haematodus caeruleiceps	9	LV 49											X	X		
107	Trichoglossus haematodus caeruleiceps	10	LV 49											X	X		
108	Trichoglossus rubritorquis	35	LV 84					X						X			
109	Triclaria malachitacea	3	LV 735	X				X									
110	Triclaria malachitacea (egg twins)	0	LV 735													X	
			Total	21	19	10	1	8	9	3	2	4	5	23	27	23	3

No proper fatty liver: Poor amount of fat stored in the hepatocytes according to the age
Retarded fatty liver: Fat vacuoles in the hepatocytes that should have been used, according to the age. Probably delayed physiologic fatty liver
Fatty liver degeneration: Vaculation, atrophy or hypotrophy of hepatocytes with fat vacuoles
Hepatitis: Increased numbers of inflammatory cells between the hepatic sinusoids and distended Kupffer's cells with phagocytized particles
Hepatosis:Vacuolated, atrophic or hypotrophic hepatocytes. Certain degree of hepatitis can be seen
Necrosis: Hepatocytes with nuclei completely broken and lost membranes. Loss of normal hepatic architecture
Haemosiderosis: following Turnbull's blue and H-E stain, presence of iron inside of the hepatocytes (estimated 1/4-1/3) and Kupffer's cells, without hepatosis and / or inflammatory reaction
Haemochromatosis: following Turnbull's blue and H-E stain,presence of iron inside of of the hepatocytes(estimated 1/3-1/2) and Kupffer's cells,with hepatosis and / or inflammatory reaction
Anaemia: low number of erythrocytes in the hepatic vessels or estimated 20% of immature erythrocytes
Poor extramedullary haematopoiesis: low number of haematopoietic foci between de hepatic tissue, according to age
Retarded extramedullary haematopoiesis: presence of haematopoietic foci between the hepatic tissue that for the age should not be there
Normal liver: no abnormalities found in the hepatocytes, surrounding conective tissue and vascularization
Autolytic liver: erythrocytes with broken nuclei, brownish discoloration of the cytoplasma and damaged hepatocytes (broken nuclei / membranes)
Not examined liver: liver not cut in the histological processing

Table 16. Lung and Airsac Findings

ID No.	Species, subspecies	Age (days)	Code of origin	Lung					Other findings in lung and airsacs
				Feed inside the trachea	Feed aspiration	Pneumonia	Anaemia	Airsacculitis	
7	Amazona ochrocephala nattereri	11	LV 876					X	
8	Amazona oratrix tresmariae	3	LV 852						Still fluid in parabronchi
22	Ara ararauna	2	LV 626				X		
23	Ara ararauna	2	LV 626	X					
29	Ara glaucogularis	10	LV 600						Oedema in parabronchi
30	Ara glaucogularis	0	LV 623						Yolk embolism
33	Ara macao	2	LV 601	X					
40	Aratinga solstitialis	5	LV 170						Pneumonic dystrophy
41	Aratinga solstitialis	7	LV 171	X	X				
58	Diopsittaca nobilis nobilis	4	LV 689	X					
60	Eclectus roratus roratus	19	AS 124						Bacteria in vessels
63	Eolophus roseicapilla	22	LV 474						Bacteria in vessels
64	Eos squamata riciniata	6	LV 57	X	X				
69	Nymphicus hollandicus	21	DF 1066			X			
71	Pionites leucogaster leucogaster	4	LV 706		X				
75	Pionus tumultuosus	1	LV 703	X					
77	Pionus tumultuosus	3	LV 703		X	X			
78	Poicephalus robustus fuscicollis	18	LV 378		X				
79	Poicephalus robustus fuscicollis	1	LV 383			X			
83	Poicephalus rufiventris	15	LV 376		X				
89	Primolius couloni	15	LV 671		X	X			
90	Primolius couloni	17	LV 671			X			
94	Primolius maracana	15	LV 419			X			
95	Primolius maracana	3	LV 423				X		Still fluids in the airsacs
98	Psittacula cyanocephala	18	LV 218					X	
109	Triclaria malachitacea	3	LV 735			X			
			Total	6	7	7	2	2	

In the birds not listed, the respiratory tract either did not show obvious lesions, was autolytic, or was not examined

Feed inside the trachea: following PAS-stain, feed particles in the lumen of the trachea

Feed aspiration: following PAS-stain, feed particles (and sometimes bacteria) inside the lung airways

Pneumonia: increased presence of inflammatory cells in the pulmonary tissue

Anaemia: low number of erythrocytes in the pulmonary vessels or estimated 20% of immature erythrocytes

Airsacculitis: thickened airsacs infiltrated with inflammatory cells

Table 18: Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney													Other findings
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined		
1	Amazona amazonica	0	LV 850											X			
2	Amazona amazonica	0	LV 850										X				
3	Amazona amazonica	18	LV 850											X			Myelocytomatosis
4	Amazona auropalliata	0	LV 844											X			
5	Amazona autumnalis salvini	0	LV 644												X		
6	Amazona barbadensis	0	LV 784											X			
7	Amazona ochrocephala nattereri	11	LV 876	X													
8	Amazona oratrix tresmariae	3	LV 852										X				
9	Amazona pretrei	0	LV 791											X			
10	Amazona rhodocorytha	0	LV 1017										X				
11	Amazona rhodocorytha	0	LV 576											X			
12	Amazona ventralis	0	AM 372											X			
13	Amazona vinacea	10	LV 1012	X								X					
14	Amazona xanthops	5	LV 820	X							X						
15	Amazona xanthops	7	LV 820										X				

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney													
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined	Other findings
16	Ara ararauna	0	LV 587										X			
17	Ara ararauna	0	LV 605	X												
18	Ara ararauna	1	LV 605		X											
19	Ara ararauna	0	LV 626											X		
20	Ara ararauna	0	LV 626		X											
21	Ara ararauna	0	LV 626										X			
22	Ara ararauna	2	LV 626							X						
23	Ara ararauna	2	LV 626							X	X					
24	Ara glaucogularis	2	LV 588		X											
25	Ara glaucogularis	3	LV 592	X							X					
26	Ara glaucogularis	0	LV 600							X						
27	Ara glaucogularis	3	LV 600								X		X			
28	Ara glaucogularis	3	LV 600													Glomerulopathy
29	Ara glaucogularis	10	LV 600									X	X			
30	Ara glaucogularis	0	LV 623											X		

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney													Other findings
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined		
31	Ara macao	0	LV 601											X			
32	Ara macao	0	LV 601												X		
33	Ara macao	2	LV 601														Mesonephros
34	Ara macao	0	LV 630												X		
35	Ara macao	0	LV 648											X			
36	Ara macao	8	LV 648	X													
37	Ara rubrogenys	3	LV 658								X		X				
38	Ara severus	3	LV 199B									X	X				
39	Ara severus	17	LV 199B									X	X				
40	Aratinga solstitialis	5	LV 170									X	X				
41	Aratinga solstitialis	7	LV 171	X													
42	Aratinga solstitialis	11	LV 171									X	X				
43	Aratinga solstitialis	8	LV 173									X	X				
44	Aratinga solstitialis	14	LV 173									X	X				
45	Cacatua leadbeateri	4	LV 34										X				

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney													Other findings
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined	
46	Cacatua pastinator	0	LV 39											X		
47	Cacatua sulphurea citrinocristata	0	LV 47			X										
48	Cyclopsitta diophthalma	4	LV 947										X			
49	Deroptyus accipitrinus fuscifrons	19	LV 679	X				X				X				
50	Diopsittaca nobilis cumanensis	5	AM 378		X		X									
51	Diopsittaca nobilis cumanensis	5	AM 378		X		X			X						
52	Diopsittaca nobilis cumanensis	0	LV 698											X		
53	Diopsittaca nobilis cumanensis	6	LV 698										X			
54	Diopsittaca nobilis cumanensis	12	LV 698	X								X				
55	Diopsittaca nobilis nobilis	3	AM 377										X			
56	Diopsittaca nobilis nobilis	8	AM 377		X					X						
57	Diopsittaca nobilis nobilis	4	LV 689		X											
58	Diopsittaca nobilis nobilis	4	LV 689										X			
59	Diopsittaca nobilis nobilis	25	LV 689	X								X				
60	Eclectus roratus roratus	19	AS 124	X		X						X				

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney													
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined	Other findings
61	Eclectus roratus solomonensis	11	LV 262							X		X				
62	Eolophus roseicapilla	0	LV 474											X		
63	Eolophus roseicapilla	22	LV 474	X												
64	Eos squamata riciniata	6	LV 57	X												
65	Forpus passerinus	0	LV 1000											X		
66	Loriculus vernalis	15	LV 991									X	X			
67	Loriculus vernalis	18	LV 991									X	X			
68	Neophema splendida	18	AU 2							X						
69	Nymphicus hollandicus	21	DF 1066	X												
70	Orthopsittaca manilata	0	AM 380												X	
71	Pionites leucogaster leucogaster	4	LV 706										X			
72	Pionites leucogaster leucogaster	0	LV 715											X		
73	Pionites melanocephalus	9	AM 253									X	X			
74	Pionus menstruus	3	LV 915										X			
75	Pionus tumultuosus	1	LV 703	X												

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney												Other findings
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined	
76	Pionus tumultuosus	2	LV 703								X		X			
77	Pionus tumultuosus	3	LV 703							X	X					
78	Poicephalus robustus fuscicollis	18	LV 378												X	
79	Poicephalus robustus fuscicollis	1	LV 383										X			
80	Poicephalus rufiventris	8	AF 5									X	X			
81	Poicephalus rufiventris	8	LV 376									X	X			
82	Poicephalus rufiventris	8	LV 376										X			
83	Poicephalus rufiventris	15	LV 376		X											
84	Poicephalus rufiventris	19	LV 376		X		X					X				
85	Poicephalus rufiventris	0	LV 380											X		
86	Poicephalus rufiventris	12	LV 380									X	X			
87	Primolius auricollis	2	AM 382										X			
88	Primolius couloni	2	AM 379										X			
89	Primolius couloni	15	LV 671									X				Interstitial oedema
90	Primolius couloni	17	LV 671									X	X			

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney												Other findings
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedulary granulopoiesis	Normal	Autolytic	Not examined	
91	Primolius couloni	21	LV 671									X	X			
92	Primolius maracana	0	LV 419											X		
93	Primolius maracana	0	LV 419	X												
94	Primolius maracana	15	LV 419				X					X				
95	Primolius maracana	3	LV 423								X		X			
96	Psittacula alexandri abbotti	2	LV 236							X						
97	Psittacula cyanocephala	10	LV 218									X	X			
98	Psittacula cyanocephala	18	LV 218									X	X			
99	Psittacula krameri manillensis	6	LV 217	X												
100	Psittacus erithacus timneh	11	LV 514									X	X			
101	Pyrrhura lepida	0	LV 284												X	
102	Pyrrhura perlata	2	LV 145										X			
103	Rhynchopsitta pachyrhyncha	3	LV 678								X		X			
104	Trichoglossus capistratus	4	LV 98										X			

Table 18 (continued): Histological Evaluation of the Kidney

ID No.	Species, subspecies	Age (days)	Code of origin	Kidney												Other findings
				Tubulonephrosis	Chronic tubulonephr. (mineralization)	Tubulonecrosis	Glomerulonephritis	PAS + glomerulopathy	Immature glomeruli (according to age)	Anaemic	Poor extramedullary granulopoiesis	Retarded extramedullary granulopoiesis	Normal	Autolytic	Not examined	
105	Trichoglossus haematodus caeruleiceps	0	LV 49											X		
106	Trichoglossus haematodus caeruleiceps	9	LV 49									X	X			
107	Trichoglossus haematodus caeruleiceps	10	LV 49									X	X			
108	Trichoglossus rubritorquis	35	LV 84						X							
109	Triclaria malachitacea	3	LV 735										X			
110	Triclaria malachitacea (egg twins)	0	LV 735											X		
			Total	17	9	2	4	1	1	9	9	29	45	20	6	

Tubulonephrosis: vacuolated, atrophic or hypotrophic tubular cells, distended tubules and sometimes inflammatory reaction
Chronic tubulonephrosis: tubulonephrosis and tubular mineralization
Tubulonecrosis: necrotic tubular cells (eosinophilic cytoplasm and nuclear pyknosis)
Glomerulonephritis: adhesions of the parietal membrane or visceral membrane of the glomeruli
PAS + glomerulopathy: following PAS-stain, thickened glomerular membranes
Immature glomeruli: according to age, high estimation of closed capillary loops of the glomeruli
Anaemic kidney: low number of erythrocytes in the renal vessels or estimated 20% of immature erythrocytes
Poor extramedullary haematopoiesis: low number of granulopoietic foci between the renal tissue, according to age
Retarded extramedullary haematopoiesis: presence of granulopoietic foci between the renal tissue that for the age should not be there
Normal kidney: no abnormalities found in the tubular cells, glomeruli, surrounding conective tissue and vascularization
Autolytic kidney: erythrocytes with broken nuclei, brownish discoloration of the cytoplasma and damaged tubular cells (broken nuclei / membranes)
Not examined kidney: kidney not cut in the histological processing

Table 19: Histological Evaluation of the Heart

ID No.	Species, subspecies	Age (days)	Code of origin	Heart					
				Myocarditis	Myodegeneration	Interstitial oedema	Normal	Autolytic	Not examined
1	Amazona amazonica	0	LV 850						X
2	Amazona amazonica	0	LV 850						X
3	Amazona amazonica	18	LV 850					X	
4	Amazona auropalliata	0	LV 844					X	
5	Amazona autumnalis salvini	0	LV 644					X	
6	Amazona barbadensis	0	LV 784					X	
7	Amazona ochrocephala nattereri	11	LV 876		X				
8	Amazona oratrix tresmariae	3	LV 852					X	
9	Amazona pretrei	0	LV 791					X	
10	Amazona rhodocorytha	0	LV 1017		X				
11	Amazona rhodocorytha	0	LV 576					X	
12	Amazona ventralis	0	AM 372					X	
13	Amazona vinacea	10	LV 1012				X		
14	Amazona xanthops	5	LV 820				X		
15	Amazona xanthops	7	LV 820					X	
16	Ara ararauna	0	LV 587					X	
17	Ara ararauna	0	LV 605		X				
18	Ara ararauna	1	LV 605		X				
19	Ara ararauna	0	LV 626						X
20	Ara ararauna	0	LV 626					X	
21	Ara ararauna	0	LV 626		X	X			
22	Ara ararauna	2	LV 626				X		
23	Ara ararauna	2	LV 626		X				
24	Ara glaucogularis	2	LV 588				X		
25	Ara glaucogularis	3	LV 592		X				
26	Ara glaucogularis	0	LV 600					X	
27	Ara glaucogularis	3	LV 600				X		
28	Ara glaucogularis	3	LV 600		X				
29	Ara glaucogularis	10	LV 600			X			
30	Ara glaucogularis	0	LV 623					X	
31	Ara macao	0	LV 601					X	
32	Ara macao	0	LV 601					X	

Table 19 (continued): Histological Evaluation of the Heart

ID No.	Species, subspecies	Age (days)	Code of origin	Heart					
				Myocarditis	Myodegeneration	Interstitial oedema	Normal	Autolytic	Not examined
33	Ara macao	2	LV 601					X	
34	Ara macao	0	LV 630					X	
35	Ara macao	0	LV 648					X	
36	Ara macao	8	LV 648		X				
37	Ara rubrogenys	3	LV 658				X		
38	Ara severus	3	LV 199B			X			
39	Ara severus	17	LV 199B				X		
40	Aratinga solstitialis	5	LV 170				X		
41	Aratinga solstitialis	7	LV 171	X					
42	Aratinga solstitialis	11	LV 171		X				
43	Aratinga solstitialis	8	LV 173				X		
44	Aratinga solstitialis	14	LV 173				X		
45	Cacatua leadbeateri	4	LV 34			X			
46	Cacatua pastinator	0	LV 39					X	
47	Cacatua sulphurea citrinocristata	0	LV 47				X		
48	Cyclopsitta diophthalma	4	LV 947						X
49	Deroptyus accipitrinus fuscifrons	19	LV 679		X	X			
50	Diopsittaca nobilis cumanensis	5	AM 378	X					
51	Diopsittaca nobilis cumanensis	5	AM 378		X				
52	Diopsittaca nobilis cumanensis	0	LV 698					X	
53	Diopsittaca nobilis cumanensis	6	LV 698				X		
54	Diopsittaca nobilis cumanensis	12	LV 698		X				
55	Diopsittaca nobilis nobilis	3	AM 377					X	
56	Diopsittaca nobilis nobilis	8	AM 377				X		
57	Diopsittaca nobilis nobilis	4	LV 689			X			
58	Diopsittaca nobilis nobilis	4	LV 689				X		
59	Diopsittaca nobilis nobilis	25	LV 689		X				
60	Eclectus roratus roratus	19	AS 124	Thin left ventricle wall					
61	Eclectus roratus solomonensis	11	LV 262			X			
62	Eolophus roseicapilla	0	LV 474					X	
63	Eolophus roseicapilla	22	LV 474				X		
64	Eos squamata riciniata	6	LV 57		X				

Table 19 (continued) Histological Evaluation of the Heart

ID No.	Species, subspecies	Age (days)	Code of origin	Heart					
				Myocarditis	Myodegeneration	Interstitial oedema	Normal	Autolytic	Not examined
65	Forpus passerinus	0	LV 1000					X	
66	Loriculus vernalis	15	LV 991			X			
67	Loriculus vernalis	18	LV 991			X			
68	Neophema splendida	18	AU 2						X
69	Nymphicus hollandicus	21	DF 1066		X				
70	Orthopsittaca manilata	0	AM 380					X	
71	Pionites leucogaster leucogaster	4	LV 706		X				
72	Pionites leucogaster leucogaster	0	LV 715					X	
73	Pionites melanocephalus	9	AM 253						X
74	Pionus menstruus	3	LV 915		X				
75	Pionus tumultuosus	1	LV 703		X				
76	Pionus tumultuosus	2	LV 703				X		
77	Pionus tumultuosus	3	LV 703				X		
78	Poicephalus robustus fuscicollis	18	LV 378				X		
79	Poicephalus robustus fuscicollis	1	LV 383				X		
80	Poicephalus rufiventris	8	AF 5				X		
81	Poicephalus rufiventris	8	LV 376			X			
82	Poicephalus rufiventris	8	LV 376				X		
83	Poicephalus rufiventris	15	LV 376				X		
84	Poicephalus rufiventris	19	LV 376				X		
85	Poicephalus rufiventris	0	LV 380					X	
86	Poicephalus rufiventris	12	LV 380				X		
87	Primolius auricollis	2	AM 382				X		
88	Primolius couloni	2	AM 379				X		
89	Primolius couloni	15	LV 671				X		
90	Primolius couloni	17	LV 671			X			
91	Primolius couloni	21	LV 671				X		
92	Primolius maracana	0	LV 419					X	
93	Primolius maracana	0	LV 419				X		
94	Primolius maracana	15	LV 419	X					
95	Primolius maracana	3	LV 423			X			
96	Psittacula alexandri abbotti	2	LV 236					X	

Table 19 (continued): Histological Evaluation of the Heart

ID No.	Species, subspecies	Age (days)	Code of origin	Heart					
				Myocarditis	Myodegeneration	Interstitial oedema	Normal	Autolytic	Not examined
97	Psittacula cyanocephala	10	LV 218				X		
98	Psittacula cyanocephala	18	LV 218				X		
99	Psittacula krameri manillensis	6	LV 217		X				
100	Psittacus erithacus timneh	11	LV 514		X				
101	Pyrrhura lepida	0	LV 284						X
102	Pyrrhura perlata	2	LV 145				X		
103	Rhynchopsitta pachyrhyncha	3	LV 678			X			
104	Trichoglossus capistratus	4	LV 98				X		
105	Trichoglossus haematodus caeruleiceps	0	LV 49						X
106	Trichoglossus haematodus caeruleiceps	9	LV 49			X			
107	Trichoglossus haematodus caeruleiceps	10	LV 49				X		
108	Trichoglossus rubritorquis	35	LV 84	X					
109	Triclaria malachitacea	3	LV 735						X
110	Triclaria malachitacea (egg twins)	0	LV 735						X
			Total	4	21	14	34	28	10

Myocarditis: myocardial tissue infiltrated by inflammatory cells
Myodegeneration of the heart: contraction band formation, cross-striation loss, swelling and hyalinization which can progress to segmental fragmentation of myofibers
Interstitial oedema: oedematous fluid (slightly eosinophilic) separating the myocardial fibers
Normal heart: no abnormalities found in the myocardial cells, epicardium and endocardium, surrounding conective tissue, vascularization and Purkinje fibres
Autolytic heart: erythrocytes with broken nuclei, brownish discoloration of the cytoplasma and damaged myocardial cells (broken nuclei / membranes)
Not examined heart: heart not cut in the histological processing

Table 20: Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
1	Amazona amazonica	0	LV 850			X				X	X		X					X	
2	Amazona amazonica	0	LV 850							X							X		
3	Amazona amazonica	18	LV 850	X					X		X				X	X			
4	Amazona auropalliata	0	LV 844			X							X					X	
5	Amazona autumnalis salvini	0	LV 644			X							X				X		
6	Amazona barbadensis	0	LV 784															X	
7	Amazona ochrocephala nattereri	11	LV 876	X					X	X	X				X		X		
8	Amazona oratrix tresmariae	3	LV 852								X					X			
9	Amazona pretrei	0	LV 791															X	
10	Amazona rhodocorytha	0	LV 1017	X						X	X						X		
11	Amazona rhodocorytha	0	LV 576							X		X					X		
12	Amazona ventralis	0	AM 372															X	
13	Amazona vinacea	10	LV 1012							X		X			X	X			
14	Amazona xanthops	5	LV 820	X							X					X			
15	Amazona xanthops	7	LV 820	X	X	X			X	X	X		X		X		X		

Table 20 (continued): Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
16	Ara ararauna	0	LV 587	X						X	X							X	
17	Ara ararauna	0	LV 605	X						X	X	X		X			X		
18	Ara ararauna	1	LV 605	X		X				X	X		X	X			X		
19	Ara ararauna	0	LV 626															X	
20	Ara ararauna	0	LV 626							X		X					X		
21	Ara ararauna	0	LV 626	X			X			X		X		X			X		
22	Ara ararauna	2	LV 626	X				X		X	X						X		
23	Ara ararauna	2	LV 626	X				X		X							X		
24	Ara glaucogularis	2	LV 588	X						X	X	X		X			X		
25	Ara glaucogularis	3	LV 592	X							X						X		
26	Ara glaucogularis	0	LV 600							X							X		
27	Ara glaucogularis	3	LV 600	X						X	X			X			X		
28	Ara glaucogularis	3	LV 600	X						X	X			X			X		
29	Ara glaucogularis	10	LV 600	X			X		X	X	X	X		X	X		X		
30	Ara glaucogularis	0	LV 623							X								X	

Table 20 (continued): Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
31	Ara macao	0	LV 601															X	
32	Ara macao	0	LV 601											X			X		
33	Ara macao	2	LV 601	X						X	X	X					X		
34	Ara macao	0	LV 630									X		X			X		
35	Ara macao	0	LV 648															X	
36	Ara macao	8	LV 648							X	X						X		
37	Ara rubrogenys	3	LV 658	X						X	X						X		
38	Ara severus	3	LV 199B	X						X	X								X
39	Ara severus	17	LV 199B	X					X	X	X	X		X	X		X		
40	Aratinga solstitialis	5	LV 170	X						X	X						X		
41	Aratinga solstitialis	7	LV 171	X						X	X					X			
42	Aratinga solstitialis	11	LV 171	X		X			X	X			X	X	X		X		
43	Aratinga solstitialis	8	LV 173	X	X				X	X	X				X	X			
44	Aratinga solstitialis	14	LV 173	X					X	X	X				X	X			
45	Cacatua leadbeateri	4	LV 34	X						X	X					X			

Table 20 (continued): Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
46	Cacatua pastinator	0	LV 39														X		
47	Cacatua sulphurea citrinocristata	0	LV 47	X		X				X	X		X	X			X		
48	Cyclopsitta diophthalma	4	LV 947	X						X	X					X			
49	Deroptyus accipitrinus fuscifrons	19	LV 679							X					X		X		
50	Diopsittaca nobilis cumanensis	5	AM 378							X	X	X			X		X		
51	Diopsittaca nobilis cumanensis	5	AM 378	X						X		X			X		X		
52	Diopsittaca nobilis cumanensis	0	LV 698															X	
53	Diopsittaca nobilis cumanensis	6	LV 698	X						X	X						X		
54	Diopsittaca nobilis cumanensis	12	LV 698							X					X		X		
55	Diopsittaca nobilis nobilis	3	AM 377	X						X	X						X		
56	Diopsittaca nobilis nobilis	8	AM 377	X							X						X		
57	Diopsittaca nobilis nobilis	4	LV 689	X						X	X					X			
58	Diopsittaca nobilis nobilis	4	LV 689	X						X	X					X			
59	Diopsittaca nobilis nobilis	25	LV 689	X					X	X	X				X		X		
60	Eclectus roratus roratus	19	AS 124	X					X	X	X				X	X			

Table 20 (continued): Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
61	Eclectus roratus solomonensis	11	LV 262	X				X	X	X	X				X	X			
62	Eolophus roseicapilla	0	LV 474							X						X			
63	Eolophus roseicapilla	22	LV 474													X			
64	Eos squamata riciniata	6	LV 57	X						X	X	X			X	X			
65	Forpus passerinus	0	LV 1000															X	
66	Loriculus vernalis	15	LV 991	X					X	X	X				X		X		
67	Loriculus vernalis	18	LV 991	X					X	X	X	X			X		X		
68	Neophema splendida	18	AU 2													X			
69	Nymphicus hollandicus	21	DF 1066														X		
70	Orthopsittaca manilata	0	AM 380									X					X		
71	Pionites leucogaster leucogaster	4	LV 706							X							X		
72	Pionites leucogaster leucogaster	0	LV 715			X							X					X	
73	Pionites melanocephalus	9	AM 253	X	X				X	X					X				X
74	Pionus menstruus	3	LV 915	X						X	X	X					X		
75	Pionus tumultuosus	1	LV 703			X				X			X				X		

Table 20 (continued): Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
91	Primolius couloni	21	LV 671							X	X	X		X	X		X		
92	Primolius maracana	0	LV 419															X	
93	Primolius maracana	0	LV 419	X		X	X			X	X	X	X	X			X		
94	Primolius maracana	15	LV 419	X		X		X	X	X	X		X		X		X		
95	Primolius maracana	3	LV 423	X							X					X			
96	Psittacula alexandri abbotti	2	LV 236			X				X			X				X		
97	Psittacula cyanocephala	10	LV 218	X					X	X	X				X	X			
98	Psittacula cyanocephala	18	LV 218							X					X		X		
99	Psittacula krameri manillensis	6	LV 217							X	X	X			X		X		
100	Psittacus erithacus timneh	11	LV 514	X					X	X	X				X	X			
101	Pyrrhura lepida	0	LV 284																X
102	Pyrrhura perlata	2	LV 145	X						X	X					X			
103	Rhynchopsitta pachyrhyncha	3	LV 678	X						X	X						X		
104	Trichoglossus capistratus	4	LV 98	X						X	X						X		
105	Trichoglossus haematodus caeruleiceps	0	LV 49															X	

Table 20 (continued): Histologic Evaluation of the Haematopoietic Tissue

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of extramedullary erythropoiesis				Immature erythrocytes in peripheral blood	Retarded e. Erythropoiesis	Presence of extramedullary granulopoiesis					Retarded e. Granulopoiesis	Bone marrow colonization (according to age)			
				Liver	Kidney	Around Sp. cord	Other organs			Kidney	Liver	Spleen	Around Sp. cord	Other organs		Poor	Well	Autolytic	Not examined
106	Trichoglossus haematodus caeruleiceps	9	LV 49	X				X	X	X	X				X	X			
107	Trichoglossus haematodus caeruleiceps	10	LV 49	X					X	X	X	X			X				X
108	Trichoglossus rubritorquis	35	LV 84								X	X			X		X		
109	Triclaria malachitacea	3	LV 735							X	X					X			
110	Triclaria malachitacea (egg twins)	0	LV 735																X
							Total	6	24					Total	38	23	67	15	5

X Retarded extramedullary granulopoiesis in that organ

Immature erythrocytes in peripheral blood: presence of estimated 20% of immature erythrocytes (elongated net-like nuclei, lack of haemoglobin, rounded nuclei)
Retarded extramedullary erythropoiesis: presence of erythropoietic foci extramedullary that for the age should not be there
Retarded extramedullary granulopoiesis: presence of granulopoietic foci extramedullary that for the age should not be there
Poorly colonized bone marrow: estimated 50% less haematopoietic tissue that should be there, according to age
Well colonized bone marrow: estimated good amount of haematopoietic tissue, according to age
Autolytic bone marrow: erythrocytes and haematopoietic cells with broken nuclei, brownish discoloration of the cytoplasma
Not examined bone marrow: bone marrow not cut in the histological processing

Table 21: Histological Evaluation of the Central Nervous System

ID No.	Species, subspecies	Age (days)	Code of origin	Cerebrum							Spinal cord				
				Neuropil vacuolation	Neuronal vacuolar degen.	Anaemia	Immature	Normal (according to age)	Autolytic	Not examined	Neuropil vacuolation	Neuronal vacuolar degen.	Normal	Autolytic	Not examined
1	Amazona amazonica	0	LV 850						X				X		
2	Amazona amazonica	0	LV 850						X				X		
3	Amazona amazonica	18	LV 850			X								X	
4	Amazona auropalliata	0	LV 844						X					X	
5	Amazona autumnalis salvini	0	LV 644					X						X	
6	Amazona barbadensis	0	LV 784						X				X		
7	Amazona ochrocephala nattereri	11	LV 876					X			X				
8	Amazona oratrix tresmariae	3	LV 852					X					X		
9	Amazona pretrei	0	LV 791						X					X	
10	Amazona rhodocorytha	0	LV 1017					X					X		
11	Amazona rhodocorytha	0	LV 576					X						X	
12	Amazona ventralis	0	AM 372						X					X	
13	Amazona vinacea	10	LV 1012					X					X		
14	Amazona xanthops	5	LV 820							X					X
15	Amazona xanthops	7	LV 820			X	See text (page 65)						X		
16	Ara ararauna	0	LV 587						X					X	
17	Ara ararauna	0	LV 605					X					X		
18	Ara ararauna	1	LV 605			X							X		
19	Ara ararauna	0	LV 626						X					X	
20	Ara ararauna	0	LV 626							X			X		

Table 21 (continued): Histological Evaluation of the Central Nervous System

ID No.	Species, subspecies	Age (days)	Code of origin	Cerebrum							Spinal cord				
				Neuropil vacuolation	Neuronal vacuolar degen.	Anaemia	Immature	Normal (according to age)	Autolytic	Not examined	Neuropil vacuolation	Neuronal vacuolar degen.	Normal	Autolytic	Not examined
41	Aratinga solstitialis	7	LV 171					X			X				
42	Aratinga solstitialis	11	LV 171					X					X		
43	Aratinga solstitialis	8	LV 173							X		X			
44	Aratinga solstitialis	14	LV 173					X			X				
45	Cacatua leadbeateri	4	LV 34					X				X			
46	Cacatua pastinator	0	LV 39						X					X	
47	Cacatua sulphurea citrinocristata	0	LV 47							X	X				
48	Cyclopsitta diophthalma	4	LV 947					X			X				
49	Deroptyus accipitrinus fuscifrons	19	LV 679					X			X				
50	Diopsittaca nobilis cumanensis	5	AM 378							X	X				
51	Diopsittaca nobilis cumanensis	5	AM 378					X				X			
52	Diopsittaca nobilis cumanensis	0	LV 698						X					X	
53	Diopsittaca nobilis cumanensis	6	LV 698					X					X		
54	Diopsittaca nobilis cumanensis	12	LV 698					X					X		
55	Diopsittaca nobilis nobilis	3	AM 377			X					X				
56	Diopsittaca nobilis nobilis	8	AM 377					X			X				
57	Diopsittaca nobilis nobilis	4	LV 689					X			X				
58	Diopsittaca nobilis nobilis	4	LV 689						X		X				
59	Diopsittaca nobilis nobilis	25	LV 689					X					X		
60	Eclectus roratus roratus	19	AS 124	Cocci in blood vessels										X	

Table 21 (continued): Histological Evaluation of the Central Nervous System

ID No.	Species, subspecies	Age (days)	Code of origin	Cerebrum							Spinal cord				
				Neuropil vacuolation	Neuronal vacuolar degen.	Anaemia	Immature	Normal (according to age)	Autolytic	Not examined	Neuropil vacuolation	Neuronal vacuolar degen.	Normal	Autolytic	Not examined
61	Eclectus roratus solomonensis	11	LV 262			X								X	
62	Eolophus roseicapilla	0	LV 474						X				X		
63	Eolophus roseicapilla	22	LV 474	X		X			Bacteria		X				
64	Eos squamata riciniata	6	LV 57					X					X		
65	Forpus passerinus	0	LV 1000						X					X	
66	Loriculus vernalis	15	LV 991					X					X		
67	Loriculus vernalis	18	LV 991	X			X						X		
68	Neophema splendida	18	AU 2					X			X				
69	Nymphicus hollandicus	21	DF 1066	X							X				
70	Orthopsittaca manilata	0	AM 380						X				X		
71	Pionites leucogaster leucogaster	4	LV 706			X							X		
72	Pionites leucogaster leucogaster	0	LV 715						X					X	
73	Pionites melanocephalus	9	AM 253	X							X				
74	Pionus menstruus	3	LV 915					X					X		
75	Pionus tumultuosus	1	LV 703					X					X		
76	Pionus tumultuosus	2	LV 703					X					X		
77	Pionus tumultuosus	3	LV 703			X							X		
78	Poicephalus robustus fuscicollis	18	LV 378		X		X								X
79	Poicephalus robustus fuscicollis	1	LV 383			X							X		
80	Poicephalus rufiventris	8	AF 5					X			X				

Table 21 (continued): Histological Evaluation of the Central Nervous System

ID No.	Species, subspecies	Age (days)	Code of origin	Cerebrum							Spinal cord				
				Neuropil vacuolation	Neuronal vacuolar degen.	Anaemia	Immature	Normal (according to age)	Autolytic	Not examined	Neuropil vacuolation	Neuronal vacuolar degen.	Normal	Autolytic	Not examined
81	Poicephalus rufiventris	8	LV 376					X			X				
82	Poicephalus rufiventris	8	LV 376					X			X				
83	Poicephalus rufiventris	15	LV 376							X		X			
84	Poicephalus rufiventris	19	LV 376				X						X		
85	Poicephalus rufiventris	0	LV 380							X				X	
86	Poicephalus rufiventris	12	LV 380					X			X				
87	Primolius auricollis	2	AM 382			X							X		
88	Primolius couloni	2	AM 379			X					X				
89	Primolius couloni	15	LV 671			X					X				
90	Primolius couloni	17	LV 671				X				X				
91	Primolius couloni	21	LV 671	X			X				X				
92	Primolius maracana	0	LV 419							X				X	
93	Primolius maracana	0	LV 419					X			X				
94	Primolius maracana	15	LV 419			X	X				X				
95	Primolius maracana	3	LV 423			X					X				
96	Psittacula alexandri abbotti	2	LV 236	Multiple haemorrhages									X		
97	Psittacula cyanocephala	10	LV 218					X						X	
98	Psittacula cyanocephala	18	LV 218					X			X				
99	Psittacula krameri manillensis	6	LV 217			X							X		
100	Psittacus erithacus timneh	11	LV 514					X			X				

Table 21 (continued): Histological Evaluation of the Central Nervous System

ID No.	Species, subspecies	Age (days)	Code of origin	Cerebrum							Spinal cord				
				Neuropil vacuolation	Neuronal vacuolar degen.	Anaemia	Immature	Normal (according to age)	Autolytic	Not examined	Neuropil vacuolation	Neuronal vacuolar degen.	Normal	Autolytic	Not examined
101	Pyrrhura lepida	0	LV 284							X			X		
102	Pyrrhura perlata	2	LV 145						X		X				
103	Rhynchopsitta pachyrhyncha	3	LV 678	Congenital dysplasia							X				
104	Trichoglossus capistratus	4	LV 98					X					X		
105	Trichoglossus haematodus caeruleiceps	0	LV 49						X					X	
106	Trichoglossus haematodus caeruleiceps	9	LV 49					X					X		
107	Trichoglossus haematodus caeruleiceps	10	LV 49					X			X				
108	Trichoglossus rubritorquis	35	LV 84				X						X		
109	Triclaria malachitacea	3	LV 735	X							X				
110	Triclaria malachitacea (egg twins)	0	LV 735							X				X	
			Total	7	1	18	7	43	23	13	38	5	42	23	3

Neuropil vacuolation: presence of vacuoles in the neuropil
Neuronal vacuolar degeneration: presence of cytoplasmatic vacuoles in the neurons
Anaemic cerebrum: low number of erythrocytes in the cerebral vessels or estimated 20% of immature erythrocytes
Immature cerebrum: birds older than 3 weeks, with still too many nerve cells in the cerebrum and / or a rather thick granular layer
Normal cerebrum: no abnormalities found in nervous tissue, surrounding connective tissue and central nervous tissue vascularization
Autolytic cerebrum: erythrocytes with broken nuclei and brownish discoloration of the cytoplasm
Not examined cerebrum: cerebrum not cut in the histological processing
Normal spinal cord: no abnormalities found in the spinal cord, surrounding connective tissue and vascularization
Autolytic spinal cord: erythrocytes with broken nuclei and brownish discoloration of the cytoplasma
Not examined spinal cord: spinal cord not cut in the histological processing

Table 22: Bacterial Findings and Evaluation of the Bacterial Infection

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of bacteria (histopathology)						Presence of inflammatory cells in the infected organ	Post-mortem microbiology of the heart		Post-mortem microbiology of the liver		Other microbiology post-mortem findings and isolated bacteria from the heart or liver	Ante-mortem findings (cloacal swabs)	Ante-mortem antibiotic treatment	Bacterial status			
				Lung	Yolk sac	Liver	Kidney	Intestine	Other organs		Gram +	Gram -	Gram +	Gram -				Local infection	Secondary inf.	Septicaemia	Not relevant
3	Amazona amazonica	18	LV 850													[illegible]	X		X		
7	Amazona ochrocephala natterer	11	LV 876							X	++	++		+++	Ps.aerug. also in lung					X	
13	Amazona vinacea	10	LV 1012										+		Streptoc. spp.	[illegible] E.coli	X				X
14	Amazona xanthops	5	LV 820				X		X	X						Umbilicus infection	X			X	
15	Amazona xanthops	7	LV 820													+++ E.coli	X			X	
22	Ara ararauna	2	LV 626		X									++	E.coli	+++ E.coli	X			X	
25	Ara glaucogularis	3	LV 592		X					X				++	E.coli	+++ E.coli	X			X	
26	Ara glaucogularis	0	LV 600		X				X										X		
27	Ara glaucogularis	3	LV 600								+		++			+++ E.coli	X			X	
28	Ara glaucogularis	3	LV 600								++										X
29	Ara glaucogularis	10	LV 600								1c.		++		Lu: 4 gram + col.						X
33	Ara macao	2	LV 601													+++ E.coli	X			X	
36	Ara macao	8	LV 648													[illegible] E.coli	X				X
37	Ara rubrogenys	3	LV 658											+	E.coli						X
38	Ara severus	3	LV 199B					X			5 c.				B- haemolytic		X			X	
39	Ara severus	17	LV 199B		X					X				Few				X			
40	Aratinga solstitialis	5	LV 170		X													X			
41	Aratinga solstitialis	7	LV 171	X						X										X	
42	Aratinga solstitialis	11	LV 171										++		Streptoc. spp.	[illegible] E.coli	X				X
43	Aratinga solstitialis	8	LV 173													+ gram -					X

Table 22 (continued): Bacterial Findings and Evaluation of the Bacterial Infection

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of bacteria (histopathology)						Presence of inflammatory cells in the infected organ	Post-mortem microbiology of the heart		Post-mortem microbiology of the liver		Other microbiology post-mortem findings and isolated bacteria from the heart or liver	Ante-mortem findings (cloacal swabs)	Ante-mortem antibiotic treatment	Bacterial status			
				Lung	Yolk sac	Liver	Kidney	Intestine	Other organs		Gram +	Gram -	Gram +	Gram -				Local infection	Secondary inf.	Septicaemia	Not relevant
44	Aratinga solstitialis	14	LV 173													Few E.coli/Pseudom	X		X		
45	Cacatua leadbeateri	4	LV 34													Few gram +					X
49	Deroptyus accipitrinus fuscifrons	19	LV 679			X				X	4 c.			++	Klebs./E.coli mucoid					X	
50	Diopsittaca nobilis cumanensis	5	AM 378							X						E.coli	X				X
55	Diopsittaca nobilis nobilis	3	AM 377													+++ E.coli	X			X	
56	Diopsittaca nobilis nobilis	8	AM 377													E.coli	X				X
57	Diopsittaca nobilis nobilis	4	LV 689													gram + + gram -	X				X
59	Diopsittaca nobilis nobilis	25	LV 689							X				+++	E.coli	Recurrent infections	X			X	
60	Eclectus roratus roratus	19	AS 124	X		X	X		X		+++		+++		Lung:+++gram + mix	Many bacteria & E.coli	X			X	
61	Eclectus roratus solomonensis	11	LV 262								2 c.					E.coli	X				X
63	Eolophus roseicapilla	22	LV 474	X		X	X		X								X			X	
64	Eos squamata riciniata	6	LV 57										Few		Streptoc. spp.		X				X
69	Nymphicus hollandicus	21	DF 1066							X								X			
73	Pionites melanocephalus	9	AM 253							X	++		++		Microc. spp.	+++ E.coli	X			X	
74	Pionus menstruus	3	LV 915										Few	1 c.							X
77	Pionus tumultuosus	3	LV 703								+++		Few		Li: Pseudom.		X			X	
78	Poicephalus robustus fuscicollis	18	LV 378										Few								X
79	Poicephalus robustus fuscicollis	1	LV 383	X	X					X										X	
80	Poicephalus rufiventris	8	AF 5								2 c.			Few							X
81	Poicephalus rufiventris	8	LV 376											++	E.coli				X		

Table 22 (continued): Bacterial Findings and Evaluation of the Bacterial Infection

ID No.	Species, subspecies	Age (days)	Code of origin	Presence of bacteria (histopathology)						Presence of inflammatory cells in the infected organ	Post-mortem microbiology of the heart		Post-mortem microbiology of the liver		Other microbiology post-mortem findings and isolated bacteria from the heart or liver	Ante-mortem findings (cloacal swabs)	Ante-mortem antibiotic treatment	Bacterial status			
				Lung	Yolk sac	Liver	Kidney	Intestine	Other organs		Gram +	Gram -	Gram +	Gram -				Local infection	Secondary inf.	Septicaemia	Not relevant
82	Poicephalus rufiventris	8	LV 376											+	E.coli						X
83	Poicephalus rufiventris	15	LV 376									Few		Few	E.coli						X
86	Poicephalus rufiventris	12	LV 380								1 c.		Few		Lu:few gram + col.	[illegible]	X		X		
89	Primolius couloni	15	LV 671								+++		+		Ascit.fl: + gram + mix	[illegible]	X			X	
90	Primolius couloni	17	LV 671	X		X					Few		Few		Ascit.fl:few gram+ mix	Enlarg.belly.Pseudom.	X			X	
94	Primolius maracana	15	LV 419		X					X						[illegible]	X	X			
96	Psittacula alexandri abbotti	2	LV 236				X													X	
97	Psittacula cyanocephala	10	LV 218				X									[illegible]	X			X	
98	Psittacula cyanocephala	18	LV 218						X	X			+					X			
107	Trichoglossus haematodus caeruleiceps	10	LV 49		X											Bacillus spp.	X	X			
109	Triclaria malachitacea	3	LV 735	X						X								X			
																	Total	7	5	21	18

No. c.: Number of colonies

Few: less than 20 colonies

+: 20 - 50 colonies

++: 50 - 100 colonies

+++: > 100 colonies

[illegible]: Ante-mortem findings / treatments 5 to 6 days before death and not considered relevant

Table 24: Histologic Evaluation of the Lymphatic Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Thymus		Cloacal Bursa				Spleen				
				Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Autolytic	Not examined
1	Amazona amazonica	0	LV 850		X				X					X
2	Amazona amazonica	0	LV 850	X					X					X
3	Amazona amazonica	18	LV 850		X				X	Myelocytomatosis				
4	Amazona auropalliata	0	LV 844		X				X					X
5	Amazona autumnalis salvini	0	LV 644		X			X						X
6	Amazona barbadensis	0	LV 784		X				X					X
7	Amazona ochrocephala nattereri	11	LV 876	X			X							X
8	Amazona oratrix tresmariae	3	LV 852	X				X			X			
9	Amazona pretrei	0	LV 791		X				X					X
10	Amazona rhodocorytha	0	LV 1017	X				X						X
11	Amazona rhodocorytha	0	LV 576		X				X					X
12	Amazona ventralis	0	AM 372		X				X					X
13	Amazona vinacea	10	LV 1012		X		X			X				
14	Amazona xanthops	5	LV 820	X					X	X				
15	Amazona xanthops	7	LV 820	X			X			X				
16	Ara ararauna	0	LV 587		X				X					X
17	Ara ararauna	0	LV 605		X			X				X		
18	Ara ararauna	1	LV 605		X			X						X
19	Ara ararauna	0	LV 626		X				X					X
20	Ara ararauna	0	LV 626		X			X				X		

Table 24 (continued): Histologic Evaluation of the Lymphatic Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Thymus		Cloacal Bursa				Spleen				
				Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Autolytic	Not examined
21	Ara ararauna	0	LV 626		X				X			X		
22	Ara ararauna	2	LV 626		X			X				X		
23	Ara ararauna	2	LV 626		X				X					X
24	Ara glaucogularis	2	LV 588	X				X				X		
25	Ara glaucogularis	3	LV 592		X				X					X
26	Ara glaucogularis	0	LV 600		X			X						X
27	Ara glaucogularis	3	LV 600		X				X					X
28	Ara glaucogularis	3	LV 600	X				X				X		
29	Ara glaucogularis	10	LV 600		X				X	X				
30	Ara glaucogularis	0	LV 623		X				X					X
31	Ara macao	0	LV 601		X				X				X	
32	Ara macao	0	LV 601		X			X						X
33	Ara macao	2	LV 601		X			X				X		
34	Ara macao	0	LV 630	X				X				X		
35	Ara macao	0	LV 648		X				X					X
36	Ara macao	8	LV 648		X	X				X				
37	Ara rubrogenys	3	LV 658		X			X						X
38	Ara severus	3	LV 199B	X				X						X
39	Ara severus	17	LV 199B	X		X				X				
40	Aratinga solstitialis	5	LV 170		X			X						X

Table 24 (continued): Histologic Evaluation of the Lymphatic Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Thymus		Cloacal Bursa				Spleen				
				Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Autolytic	Not examined
41	Aratinga solstitialis	7	LV 171		X		X							X
42	Aratinga solstitialis	11	LV 171		X	X				X				
43	Aratinga solstitialis	8	LV 173		X				X					X
44	Aratinga solstitialis	14	LV 173		X				X					X
45	Cacatua leadbeateri	4	LV 34	X					X					X
46	Cacatua pastinator	0	LV 39		X				X					X
47	Cacatua sulphurea citrinocristata	0	LV 47		X			X						X
48	Cyclopsitta diophthalma	4	LV 947		X				X					X
49	Deroptyus accipitrinus fuscifrons	19	LV 679	X		Necr.medullar lymph.					X			
50	Diopsittaca nobilis cumanensis	5	AM 378		X			X		X				
51	Diopsittaca nobilis cumanensis	5	AM 378		X			X		X				
52	Diopsittaca nobilis cumanensis	0	LV 698		X				X					X
53	Diopsittaca nobilis cumanensis	6	LV 698		X	X						X		
54	Diopsittaca nobilis cumanensis	12	LV 698		X	X								X
55	Diopsittaca nobilis nobilis	3	AM 377		X			X				X		
56	Diopsittaca nobilis nobilis	8	AM 377	X					X					X
57	Diopsittaca nobilis nobilis	4	LV 689		X				X					X
58	Diopsittaca nobilis nobilis	4	LV 689	X				X				X		
59	Diopsittaca nobilis nobilis	25	LV 689		X			X			X			
60	Eclectus roratus roratus	19	AS 124	X		X	Involution?				X			

Table 24 (continued): Histologic Evaluation of the Lymphatic Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Thymus		Cloacal Bursa				Spleen				
				Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Autolytic	Not examined
61	Eclectus roratus solomonensis	11	LV 262		X				X					X
62	Eolophus roseicapilla	0	LV 474		X				X					X
63	Eolophus roseicapilla	22	LV 474	X		X	Involution?			X				
64	Eos squamata riciniata	6	LV 57		X	X				X				
65	Forpus passerinus	0	LV 1000		X				X					X
66	Loriculus vernalis	15	LV 991		X				X					X
67	Loriculus vernalis	18	LV 991		X			X		X				
68	Neophema splendida	18	AU 2		X	X				X	Some necrosis			
69	Nymphicus hollandicus	21	DF 1066	X			X				X			
70	Orthopsittaca manilata	0	AM 380		X		X					X		
71	Pionites leucogaster leucogaster	4	LV 706		X		X							X
72	Pionites leucogaster leucogaster	0	LV 715		X				X					X
73	Pionites melanocephalus	9	AM 253		X				X					X
74	Pionus menstruus	3	LV 915	X				X				X		
75	Pionus tumultuosus	1	LV 703	X				X						X
76	Pionus tumultuosus	2	LV 703	X				X				X		
77	Pionus tumultuosus	3	LV 703	X				X				X		
78	Poicephalus robustus fuscicollis	18	LV 378	X					X	X				
79	Poicephalus robustus fuscicollis	1	LV 383		X			X						X
80	Poicephalus rufiventris	8	AF 5	X				X						X

Table 24 (continued): Histologic Evaluation of the Lymphatic Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Thymus		Cloacal Bursa				Spleen				
				Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Autolytic	Not examined
81	Poicephalus rufiventris	8	LV 376		X			X				X		
82	Poicephalus rufiventris	8	LV 376		X			X		X				
83	Poicephalus rufiventris	15	LV 376		X			X		X				
84	Poicephalus rufiventris	19	LV 376		X			X		X				
85	Poicephalus rufiventris	0	LV 380		X				X					X
86	Poicephalus rufiventris	12	LV 380		X	X				X				
87	Primolius auricollis	2	AM 382		X			X				X		
88	Primolius couloni	2	AM 379	X				X				X		
89	Primolius couloni	15	LV 671	X				X				X		
90	Primolius couloni	17	LV 671		X		X					X		
91	Primolius couloni	21	LV 671	X				X		X				
92	Primolius maracana	0	LV 419		X				X					X
93	Primolius maracana	0	LV 419	X					X			X		
94	Primolius maracana	15	LV 419		X	X				X				
95	Primolius maracana	3	LV 423		X			X				X		
96	Psittacula alexandri abbotti	2	LV 236		X				X					X
97	Psittacula cyanocephala	10	LV 218		X	X								X
98	Psittacula cyanocephala	18	LV 218		X	X				X				
99	Psittacula krameri manillensis	6	LV 217		X	X				X				
100	Psittacus erithacus timneh	11	LV 514		X				X					X

Table 24 (continued): Histologic Evaluation of the Lymphatic Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Thymus		Cloacal Bursa				Spleen				
				Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Not examined	Poorly colonised	Colonised, but few mature lymph.	Normal	Autolytic	Not examined
101	Pyrrhura lepida	0	LV 284		X				X					X
102	Pyrrhura perlata	2	LV 145		X				X					X
103	Rhynchopsitta pachyrhyncha	3	LV 678		X				X			X		
104	Trichoglossus capistratus	4	LV 98		X			X				X		
105	Trichoglossus haematodus caeruleiceps	0	LV 49		X				X					X
106	Trichoglossus haematodus caeruleiceps	9	LV 49		X			X				X		
107	Trichoglossus haematodus caeruleiceps	10	LV 49		X	X						X		
108	Trichoglossus rubritorquis	35	LV 84		X		X				X			
109	Triclaria malachitacea	3	LV 735		X			X						X
110	Triclaria malachitacea (egg twins)	0	LV 735		X				X					X
			Total	28	82	15	9	41	44	22	6	26	1	54

Normal thymus: Thymus well colonised with precusor cells and mature lymphocytes
Not examined thymus: Thymus not collected or not cut in the histological processing
Poorly colonised cloacal bursa: Cloacal bursa poorly colonised with precusor cells and mature lymphocytes
Colonised cloacal bursa, but few mature lymphocytes: Cloacal bursa well colonised by precusor cells, but poorly colonised with mature lymphocytes
Normal cloacal bursa: Cloacal bursa well colonised with precusor cells and mature lymphocytes
Not examined cloacal bursa: Cloacal bursa not cut in the histological processing
Poorly colonised spleen: Spleen poorly colonised with precusor cells and mature lymphocytes
Colonised spleen, but few mature lymphocytes: Spleen well colonised by precusor cells, but poorly colonised with mature lymphocytes
Normal spleen: Spleen well colonised with precusor cells and mature lymphocytes
Autolytic spleen: erythrocytes and haematopoietic cells with broken nuclei, brownish discoloration of the cytoplasma
Not examined spleen: Spleen not cut in the histological processing

Table 25: Histopathological Findings in Other Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Parathyroid gland	Adrenal gland	Musculoskeletal system	Skin
17	Ara ararauna	0	LV 605				Subuctaneous oedema
41	Aratinga solstitialis	7	LV 171				Focal purulent dermatitis
50	Diopsittaca nobilis cumanensis	5	AM 378		Degeneration		
59	Diopsittaca nobilis nobilis	25	LV 689			Poor bone mineralisation	
63	Eolophus roseicapilla	22	LV 474		Degeneration of medullary cells		
69	Nymphicus hollandicus	21	DF 1066		Degeneration	Metabolic bone disease	
75	Pionus tumultuosus	1	LV 703	Degeneration			
78	Poicephalus robustus fuscicollis	18	LV 378		Degeneration of medullary cells		
86	Poicephalus rufiventris	12	LV 380		Degeneration		
89	Primolius couloni	15	LV 671			Myodegeneration	
91	Primolius couloni	21	LV 671		Degeneration of medullary cells	Metabolic bone disease	
104	Trichoglossus capistratus	4	LV 98		Extravasations		

In the birds not listed, the organs either did not show obvious lesions, were autolytic, or not examined

Table 28: Summary Table of the Histopathological Findings in All the Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Histopathological findings																		Bacterial findings
				GB status	Thyroid gland status	Yolk sac	Fatty liver	GI tract findings	Hepatopathies	Pancreas abnormalities	Lung development status	Respiratory tract findings	Nephropathies	Heart findings	Extramedullary erythropoiesis	Extramedullary granulopoiesis	Immat. erythr. in periph. blood	Bone marrow colonisation	Cerebrum status	Spinal cord status	
1	Amazona amazonica	0	LV 850	E	NE	EU	A	A	A	NE	N	N	A	NE	N	N	No	A	A	N	No
2	Amazona amazonica	0	LV 850	H	At	EU	A	A	A	NE	N	N	N	NE	N	N	No	N	A	N	No
3	Amazona amazonica	18	LV 850	H	NE	N	P	A	N	NE	U	N	OF	A	R	R	No	P	An	A	2
4	Amazona auropalliata	0	LV 844	H	At	EU	A	A	A	NE	A	A	A	A	N	N	No	A	A	A	No
5	Amazona autumnalis salvini	0	LV 644	E	At	EU	A	A	A	A	N	N	NE	A	N	N	No	N	N	A	No
6	Amazona barbadensis	0	LV 784	E	NE	EU	A	A	A	NE	N	N	A	A	N	N	No	A	A	N	No
7	Amazona ochrocephala natterer	11	LV 876	F	Q	R	R	A	OF	N	N	As	T	MC	R	R	No	N	N	Nv	S
8	Amazona oratrix tresmariae	3	LV 852	H	At	N	NP	OFt	NP	NE	U	OF	N	A	N	N	No	P	N	N	No
9	Amazona pretrei	0	LV 791	E	At	EU	A	A	A	NE	A	A	A	A	N	N	No	A	A	A	No
10	Amazona rhodocorytha	0	LV 1017	H	H	N	P	A	N	N	N	N	N	MC	N	N	No	N	N	N	No
11	Amazona rhodocorytha	0	LV 576	E	At	EU	A	A	A	NE	NE	NE	A	A	N	N	No	N	N	A	No
12	Amazona ventralis	0	AM 372	H	At	EU	A	A	A	NE	N	N	A	A	N	N	No	A	A	A	No
13	Amazona vinacea	10	LV 1012	H	Q	N	P	OFt	Ho	N	N	N	T	N	N	R	No	P	N	N	NR
14	Amazona xanthops	5	LV 820	H	H	EU	NP	A	NP	NZ/Dx	N	N	T	N	N	N	No	P	NE	NE	S
15	Amazona xanthops	7	LV 820	H	At	N	P	NE	N	NZ/OF	N	N	N	A	R	R	No	N	An/OFt	N	S
16	Ara ararauna	0	LV 587	H	NE	EU	A	A	A	NE	A	A	N	A	N	N	No	A	A	A	No
17	Ara ararauna	0	LV 605	F	At	N	P	O	N	N	N	N	T	MC	N	N	No	N	N	N	No
18	Ara ararauna	1	LV 605	H	At	N	P	OFt	Ne	NE	N	N	CT	MC	N	N	No	N	An	N	No
19	Ara ararauna	0	LV 626	E	At	EU	P	A	N	NE	A	A	A	NE	N	N	No	A	A	A	No
20	Ara ararauna	0	LV 626	H	NE	EU	P	A	N	NE	N	N	CT	A	N	N	No	N	NE	N	No
21	Ara ararauna	0	LV 626	E	NE	EU	P	N	N	N	N	N	N	MC/IO	N	N	No	N	Nv	NE	No
22	Ara ararauna	2	LV 626	H	At	N	P	A	N	A	N	An	An	N	N	N	Y	N	An	Nv	S
23	Ara ararauna	2	LV 626	H	At	N	P	O	N	N	N	FT	An	MC	N	N	Y	N	An	Nv	No
24	Ara glaucogularis	2	LV 588	E	At	N	P	N	N	N	N	N	CT	N	N	N	No	N	N	N	No

Table 28 (continued): Summary Table of the Histopathological Findings in All the Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Histopathological findings																	Bacterial findings
				GB status	Thyroid gland status	Yolk sac	Fatty liver	GI tract findings	Hepatopathies	Pancreas abnormalities	Lung development status	Respiratory tract findings	Nephropathies	Heart findings	Extramedullary erythropoiesis	Extramedullary granulopoiesis	Immat. erythr. in periph. blood	Bone marrow colonisation	Cerebrum status	Spinal cord status	
25	Ara glaucogularis	3	LV 592	H	At	EU	NP	O/OFt	NP	N	N	N	T	MC	N	N	No	N	An	Nv	S
26	Ara glaucogularis	0	LV 600	E	At	EU	A	I	A	NE	N	N	An	A	N	N	No	N	A	A	2
27	Ara glaucogularis	3	LV 600	H	At	N	NP	OF	NP	N	N	N	N	N	N	N	No	N	NE	Nv	S
28	Ara glaucogularis	3	LV 600	H	Q	N	NP	N	NP	N	N	N	OF	MC	N	N	No	N	N	N	NR
29	Ara glaucogularis	10	LV 600	E	At	R	P	OF	N	N	N	OF	N	IO	R	R	No	N	N	Nv	NR
30	Ara glaucogularis	0	LV 623	E	At	EU	A	O	A	A	N	OF	A	A	N	N	No	A	A	A	No
31	Ara macao	0	LV 601	F	NE	EU	A	A	A	NE	A	A	A	A	N	N	No	A	NE	A	No
32	Ara macao	0	LV 601	H	At	EU	A	A	A	NE	N	N	NE	A	N	N	No	N	A	N	No
33	Ara macao	2	LV 601	H	H	N	P	O	N	NZ	NE	FT	OF	A	N	N	No	N	NE	Nv/Nd	S
34	Ara macao	0	LV 630	E	At	EU	A	NE	A	NE	NE	NE	NE	A	N	N	No	N	A	N	No
35	Ara macao	0	LV 648	E	At	EU	A	A	A	NE	A	A	A	A	N	N	No	A	A	A	No
36	Ara macao	8	LV 648	F	H	N	P	A	N	De	U	N	T	MC	N	N	No	N	N	N	NR
37	Ara rubrogenys	3	LV 658	H	At	R	NP	O	NP	NZ	NE	NE	N	N	N	N	No	N	N	N	NR
38	Ara severus	3	LV 199B	H	At	N	P	O	N	NZ/OF	NE	NE	N	IO	N	N	No	NE	N	N	S
39	Ara severus	17	LV 199B	H	At	R	R	N	R/Ho	N	N	N	N	N	R	R	No	N	N	N	L
40	Aratinga solstitialis	5	LV 170	H	At	N	NP	A	NP	N	U	OF	N	N	N	N	No	N	A	N	L
41	Aratinga solstitialis	7	LV 171	H	NE	N	P	A	N	N	U	FT/FA	T	Mi	N	N	No	P	N	Nv	S
42	Aratinga solstitialis	11	LV 171	E	NE	R	P	A	Ne	N	U	N	N	MC	R	R	No	N	N	N	NR
43	Aratinga solstitialis	8	LV 173	E	NE	N	P	A	N	N	NE	NE	N	N	R	R	No	P	NE	Nd	NR
44	Aratinga solstitialis	14	LV 173	H	At	N	R	A	R	NE	U	N	N	N	R	R	No	P	N	Nv	2
45	Cacatua leadbeateri	4	LV 34	E	NE	EU	NP	N	NP	NE	N	N	N	IO	N	N	No	P	N	Nd	NR
46	Cacatua pastinator	0	LV 39	E	NE	EU	A	A	A	NE	A	A	A	A	N	N	No	N	A	A	No
47	Cacatua sulphurea citrinocristata	0	LV 47	H	At	EU	P	A	N	N	N	N	TN	N	N	N	No	N	NE	Nv	No
48	Cyclopsitta diophthalma	4	LV 947	E	At	EU	NP	A	NP	NZ	N	N	N	NE	N	N	No	P	N	Nv	No

Table 28 (continued): Summary Table of the Histopathological Findings in All the Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Histopathological findings																	Bacterial findings
				GB status	Thyroid gland status	Yolk sac	Fatty liver	GI tract findings	Hepatopathies	Pancreas abnormalities	Lung development status	Respiratory tract findings	Nephropathies	Heart findings	Extramedullary erythropoiesis	Extramedullary granulopoiesis	Immat. erythr. in periph. blood	Bone marrow colonisation	Cerebrum status	Spinal cord status	
49	Deroptyus accipitrinus fuscifrons	19	LV 679	H	Q	N	P	A	Hs	NZ	N	N	T/G+	MC/IO	N	R	No	N	N	Nv	S
50	Diopsittaca nobilis cumanensis	5	AM 378	H	Q	N	NP	A	NP/An	NZ	N	N	CT/Gi	Mi	N	R	No	N	NE	Nv	NR
51	Diopsittaca nobilis cumanensis	5	AM 378	H	NE	N	P	N	N	De	N	N	CT/Gi/An	MC	N	R	No	N	N	Nd	No
52	Diopsittaca nobilis cumanensis	0	LV 698	H	NE	EU	A	A	A	NE	A	A	A	A	N	N	No	A	A	A	No
53	Diopsittaca nobilis cumanensis	6	LV 698	H	NE	N	P	A	FLD	NZ	N	N	N	N	N	N	No	N	N	N	No
54	Diopsittaca nobilis cumanensis	12	LV 698	F	H	N	R	A	R/An	NZ/Dx	U	N	T	MC	N	R	No	N	N	N	No
55	Diopsittaca nobilis nobilis	3	AM 377	H	At	EU	NP	A	NP	NE	N	N	N	A	N	N	No	N	An	Nv	S
56	Diopsittaca nobilis nobilis	8	AM 377	H	Q	N	R	A	R	NZ/Dx	N	N	CT/An	N	N	N	No	N	N	Nv	NR
57	Diopsittaca nobilis nobilis	4	LV 689	H	NE	N	P	N	OF	N	N	N	CT	IO	N	N	No	P	N	Nv	NR
58	Diopsittaca nobilis nobilis	4	LV 689	H	At	N	NP	A	NP	NE	N	FT	N	N	N	N	No	P	A	Nv	No
59	Diopsittaca nobilis nobilis	25	LV 689	H	H	N	R	A	R/FLD/Hi	NZ	N	N	T	MC	R	R	No	N	N	N	S
60	Eclectus roratus roratus	19	AS 124	E	NE	N	R	A	R/Ne	N	U	OF	T/TN	OF	R	R	No	P	OF	A	S
61	Eclectus roratus solomonensis	11	LV 262	H	At	R	R	OFt	R	NE	N	N	An	IO	R	R	Y	P	An	A	NR
62	Eolophus roseicapilla	0	LV 474	E	At	EU	NE	A	NE	NE	N	N	A	A	N	N	No	P	A	N	No
63	Eolophus roseicapilla	22	LV 474	E	H	N	R	A	R/Ho/Ne	N	N	OF	T	N	N	N	No	P	Nv/An/OF	Nv	S
64	Eos squamata riciniata	6	LV 57	E	NE	N	P	A	N	NE	N	FT/FA	T	MC	N	R	No	P	N	N	NR
65	Forpus passerinus	0	LV 1000	E	NE	EU	A	A	A	NE	NE	NE	A	A	N	N	No	A	A	A	No
66	Loriculus vernalis	15	LV 991	H	At	N	R	O	R	N	U	N	N	IO	R	R	No	N	N	N	No
67	Loriculus vernalis	18	LV 991	H	At	N	R	A	R/FLD	N	N	N	N	IO	R	R	No	N	Nv/I	N	No
68	Neophema splendida	18	AU 2	E	At	N	P	W	Ho/An	N	N	N	An	NE	N	N	No	P	N	Nv	No
69	Nymphicus hollandicus	21	DF 1066	E	Q	N	NP	A	FLD	N	U	P	T	MC	N	N	No	N	Nv	Nv	L
70	Orthopsittaca manilata	0	AM 380	H	At	EU	A	A	A	NE	N	N	NE	A	N	N	No	N	A	N	No
71	Pionites leucogaster leucogaster	4	LV 706	H	NE	N	P	N	FLD	N	N	FA	N	MC	N	N	No	N	An	N	No
72	Pionites leucogaster leucogaster	0	LV 715	E	At	EU	A	A	A	NE	N	N	A	A	N	N	No	A	A	A	No

Table 28 (continued): Summary Table of the Histopathological Findings in All the Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Histopathological findings																	Bacterial findings
				GB status	Thyroid gland status	Yolk sac	Fatty liver	GI tract findings	Hepatopathies	Pancreas abnormalities	Lung development status	Respiratory tract findings	Nephropathies	Heart findings	Extramedullary erythropoiesis	Extramedullary granulopoiesis	Immat. erythr. in periph. blood	Bone marrow colonisation	Cerebrum status	Spinal cord status	
73	Pionites melanocephalus	9	AM 253	H	H	N	P	O	N	NZ	U	N	N	NE	R	R	No	NE	Nv	Nv	S
74	Pionus menstruus	3	LV 915	H	At	B	NP	F	NP	NZ	NE	NE	N	MC	N	N	No	N	N	N	NR
75	Pionus tumultuosus	1	LV 703	E	At	EU	P	O	N	N	N	FT	T	MC	N	N	No	N	N	N	S
76	Pionus tumultuosus	2	LV 703	H	At	EU	NP	N	NP	N	N	N	N	N	N	N	No	N	N	N	No
77	Pionus tumultuosus	3	LV 703	H	At	N	P	I/OFt	FLD/An	NE	N	FA/P	An	N	N	N	No	N	An	N	No
78	Poicephalus robustus fuscicollis	18	LV 378	E	Q	R	P	N	N	N	U	FA	NE	N	N	R	No	N	Nd/I	NE	NR
79	Poicephalus robustus fuscicollis	1	LV 383	H	NE	N	P	A	N	N	N	P	N	N	N	N	No	N	An	N	S
80	Poicephalus rufiventris	8	AF 5	H	H	N	NE	A	NE	N	NE	NE	N	N	N	R	No	N	N	Nv	NR
81	Poicephalus rufiventris	8	LV 376	E	NE	N	P	A	Hc	N	NE	NE	N	IO	R	R	No	N	N	Nv	2
82	Poicephalus rufiventris	8	LV 376	H	Q	N	P	A	Ho/Ne/Hc	N	N	N	N	N	N	R	No	N	N	Nv	NR
83	Poicephalus rufiventris	15	LV 376	H	H	N	R	N	R	NZ	N	FA	CT	N	R	R	No	N	NE	Nd	NR
84	Poicephalus rufiventris	19	LV 376	H	H	N	P	A	FLD	N	U	N	CT/Gi	N	R	R	No	N	I	N	No
85	Poicephalus rufiventris	0	LV 380	H	NE	EU	A	A	A	NE	N	N	A	A	N	N	No	N	NE	A	No
86	Poicephalus rufiventris	12	LV 380	F	NE	N	R	A	R	N	U	N	N	N	R	R	Y	N	N	Nv	2
87	Primolius auricollis	2	AM 382	H	At	N	P	O	OF	N	N	N	N	N	N	N	No	N	An	N	No
88	Primolius couloni	2	AM 379	H	At	EU	P	I/OFt	Ho/Ne	A	N	N	N	N	N	N	No	N	An	Nv	No
89	Primolius couloni	15	LV 671	E	At	N	R	OFt	R/FLD	N	N	FA/P	OF	N	N	R	No	N	An	Nv	S
90	Primolius couloni	17	LV 671	H	NE	N	R	OFt	R/FLD	N	U	P	N	IO	R	R	No	N	I	Nv	S
91	Primolius couloni	21	LV 671	H	At	N	R	OF	R/FLD	N	N	N	N	N	N	R	No	N	Nv/I	Nv	No
92	Primolius maracana	0	LV 419	E	At	B	P	A	N	NE	N	N	A	A	N	N	No	A	NE	A	No
93	Primolius maracana	0	LV 419	H	At	EU	P	N	N	N	N	N	T	N	N	N	No	N	N	Nv	No
94	Primolius maracana	15	LV 419	E	At	R	P	A	Ne	N	U	P	Gi	Mi	R	R	Y	N	An/I	Nv	L
95	Primolius maracana	3	LV 423	H	At	N	NP	O/I	NP	N	N	An/OF	N	IO	N	N	No	P	An	Nv	No
96	Psittacula alexandri abbotti	2	LV 236	H	NE	B	NP	A	NP	NE	N	N	An	A	N	N	No	N	OF	N	S

Table 28 (continued): Summary Table of the Histopathological Findings in All the Organs

ID No.	Species, subspecies	Age (days)	Code of origin	Histopathological findings																	Bacterial findings
				GB status	Thyroid gland status	Yolk sac	Fatty liver	GI tract findings	Hepatopathies	Pancreas abnormalities	Lung development status	Respiratory tract findings	Nephropathies	Heart findings	Extramedullary erythropoiesis	Extramedullary granulopoiesis	Immat. erythr. in periph. blood	Bone marrow colonisation	Cerebrum status	Spinal cord status	
97	Psittacula cyanocephala	10	LV 218	E	At	R	P	A	Hs	NE	U	N	N	N	R	R	No	P	N	A	S
98	Psittacula cyanocephala	18	LV 218	H	Q	N	R	O	R	NE	U	As	N	N	N	R	No	N	N	Nv	L
99	Psittacula krameri manillensis	6	LV 217	H	Q	N	NP	O	NP/Ne	N	NE	NE	T	MC	N	R	No	N	An	N	No
100	Psittacus erithacus timneh	11	LV 514	F	At	N	R	A	R/Hs	NE	U	N	N	MC	R	R	No	P	N	Nv	No
101	Pyrrhura lepida	0	LV 284	H	NE	B	NE	NE	NE	NE	NE	NE	NE	NE	N	N	No	NE	NE	N	No
102	Pyrrhura perlata	2	LV 145	H	H	N	NP	NE	NP	NE	N	N	N	N	N	N	No	P	A	Nv	No
103	Rhynchopsitta pachyrhyncha	3	LV 678	H	At	R	NP	O	Ne	NZ/OF	N	N	N	IO	N	N	No	N	OF	Nv	No
104	Trichoglossus capistratus	4	LV 98	E	At	N	NP	A	NP	N	N	N	N	N	N	N	No	N	N	N	No
105	Trichoglossus haematodus caeruleiceps	0	LV 49	E	NE	EU	A	A	A	NE	N	N	A	NE	N	N	No	A	A	A	No
106	Trichoglossus haematodus caeruleiceps	9	LV 49	H	NE	N	P	O	N	N	U	N	N	IO	R	R	No	P	N	N	No
107	Trichoglossus haematodus caeruleiceps	10	LV 49	H	H	R	P	I/OF	N	N	N	N	N	N	R	R	No	NE	N	Nv	L
108	Trichoglossus rubritorquis	35	LV 84	H	At	N	P	A	Ho	N	N	N	IG	Mi	N	R	No	N	I	N	No
109	Triclaria malachitacea	3	LV 735	E	NE	N	NP	A	NP/Ho	NE	N	P	N	NE	N	N	No	P	Nv	Nv	L
110	Triclaria malachitacea (egg twins)	0	LV 735	E	NE	EU	A	A	A	NE	A	A	A	NE	N	N	No	NE	NE	A	No

See page 179 - 181 for the legend of the summary table

Abbreviations of the summary table:

General (for all categories):

- N. Normal
- A: Autolytic
- NE: Not examined

GB status:

- E: Empty
- H: Hypotrophic
- F: Full

Thyroid gland status:

- At: Athyroidism
- H: Hypothyroidism
- Q: Quiescent

Yolk sac (YS):

- EU: Early use of yolk
- R: Retarded YS
- B. Broken YS
- E: Empty YS

Fatty liver (FL):

- NP No proper FL
- R: Retarded FL
- P: Proper FL

GI tract findings:

- O: Oedema
- I: Immature intestine
- W: Worms in the intestinal lumen
- Y. Yeasts in the intestinal lumen
- OF: Other findings
- OFt: Other findings reported in the results

Hepatopathies:

- NP: No proper FL
- R: Retarded FL
- FLD: Fatty liver degeneration
- Hi: Hepatitis
- Ho: Hepatosis
- Ne: Necrosis of liver
- Hc: Haemochromatosis

- Hs Haemosiderosis
- An· Anaemic
- OF: Other findings

Pancreas abnormalities:

- NZ: No – few zymogenic granules
- Dx· Degeneration exocrinic cells
- De: Degeneration endocrinic cells

Lung development status:

- U: Underdeveloped

Respiratory tract findings:

- FT· Food in the trachea
- FA· Feed aspiration
- P: Pneumonia
- An· Anaemia
- As. Airsacculitis

Nephropathies:

- T: Tubulonephrosis
- CT. Chronic tubulonephrosis
- TN: Tubulonecrosis
- Gi: Glomerulonephritis
- G+: PAS + glomerulopathy
- IG: Immature glomerula
- An Anaemic
- OF: Other findings

Heart findings:

- Mi: Myocarditis
- MC: Myodegeneration cordis
- IO: Interstitial oedema
- OF: Other findings

Extramedullary erythropoiesis and granulopoiesis:

- R. Retarded extramedullary erythropoiesis or granulopoiesis

Immature erythroctes in the peripheral blood:

- Y. Yes
- No: No immature erythrocytes seen in peripheral blood

Bone marrow colonisation:

- P: Poorly colonised
- N: Normal for the age

Cerebrum status:

- Nv: Neuropil vacuolation
- Nd: Neuronal vacuolar degeneration
- I: Immature cerebrum
- An: Anaemia

Spinal cord status:

- Nv: Neuropil vacuolation
- Nd: Neuronal vacuolar degeneration

Bacterial findings:

- S: Septicaemia
- 2: Secondary infection
- L: Local infection
- NR: Bacteria found not relevant
- No: No bacteria found

Table 29: Glycogen Body and Thyroid Gland Status in Embryos

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid gl. Not examined	TOTAL
Empty GB	11	0	0	7	**18**
Sev. hypotr.	6	1	0	3	**10**
Mod. hypotr.	1	0	0	2	**3**
Normal GB	1	0	0	1	**2**
TOTAL	**19**	**1**	**0**	**13**	**33**

Table 30: Glycogen Body and Thyroid Gland Status in Chicks 1 - 5 days old

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid gl. Not examined	TOTAL
Empty GB	4	0	0	2	**6**
Sev. hypotr.	15	2	2	5	**24**
Mod. hypotr.	3	1	0	0	**4**
Normal GB	0	0	0	0	**0**
TOTAL	**22**	**3**	**2**	**7**	**34**

Table 29: Glycogen Body and Thyroid Gland Status in Embryos

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid gl. Not examined	TOTAL
Empty GB	11	0	0	7	18
Sev. hypotr.	6	1	0	3	10
Mod. hypotr.	1	0	0	2	3
Normal GB	1	0	0	1	2
TOTAL	19	1	0	13	33

Table 30: Glycogen Body and Thyroid Gland Status in Chicks 1 - 5 days old

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid gl. Not examined	TOTAL
Empty GB	4	0	0	2	6
Sev. hypotr.	15	2	2	5	24
Mod. hypotr.	3	1	0	0	4
Normal GB	0	0	0	0	0
TOTAL	22	3	2	7	34

Table 33: Glycogen Body and Thyroid Gland Status in Chicks 16 - 20 days old

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid gl. Not examined	TOTAL
Empty GB	1	0	1	1	3
Sev. hypotr.	1	0	2	2	5
Mod. hypotr.	1	1	0	0	2
Normal GB	0	0	0	0	0
TOTAL	3	1	3	3	10

Table 34: Glycogen Body and Thyroid Gland Status in Chicks older than 21 days old

	Athyroidism	Hypothyroidism	Quiescent thyroid	Thyroid gl. Not examined	TOTAL
Empty GB	0	1	1	0	2
Sev. hypotr.	2	0	0	0	2
Mod. hypotr.	0	1	0	0	1
Normal GB	0	0	0	0	0
TOTAL	2	2	1	0	5

ACKNOWLEDGMENTS

To **Prof. Dr. E. F. Kaleta** from the University of Gießen, for his contribution and interest in the avian medicine, and for guiding and advising me with his experience in this doctoral thesis. I am grateful to him for accepting me as an external doctorand, and for encouraging, guiding and helping me in the avian medicine.

I specially appreciate the instruction, advice and technical help of **Prof. Dr. H. Gerlach**, without which it would not have been possible to finish this project and the doctoral thesis. I would like also to thank her for a comfortable stage in Munich

Thanks to **Loro Parque Fundación** and its director, **Dr. D. Waugh**, for the financial support and technical help that made possible this study. I appreciate the opportunity of working with the organization and with the biggest parrot collection in the world

Thank you very much to **Dr. L. G. Crosta** and **Dr. M. Bürkle** for trusting me to carry out this project. Their advice, encouragement and instruction in avian medicine has been fundamental for my professional education. I truly appreciate their educational labour and their nice working team.

Prof. Dr. Dr. Schmahl from the Ludwig–Maximilians University in Munich, thanks for his technical help discussing with his chicken chicks experience the histopathological findings with Prof. Dr. H. Gerlach.

The support, encouragment and financial help of **my family** have always been there whenever necessary and helped me to go on with the project and in my professional life, thank you from the bottom of my heart.

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- F: Full

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- R: Retarded YS
- B: Broken YS
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- NP: No proper FL
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- Ho: Hepatosis
- Ne: Necrosis of liver
- Hc: Haemochromatosis

- Hs: Haemosiderosis
- An: Anaemic
- OF: Other findings

Pancreas abnormalities:

- NZ: No – few zymogenic granules
- Dx: Degeneration exocrinic cells
- De: Degeneration endocrinic cells

Lung development status:

- U: Underdeveloped

Respiratory tract findings:

- FT: Food in the trachea
- FA: Feed aspiration
- P: Pneumonia
- An: Anaemia
- As: Airsacculitis

Nephropathies:

- T: Tubulonephrosis
- CT: Chronic tubulonephrosis
- TN: Tubulonecrosis
- Gi: Glomerulonephritis
- G+: PAS + glomerulopathy
- IG: Immature glomerula
- An: Anaemic
- OF: Other findings

Heart findings:

- Mi: Myocarditis
- MC: Myodegeneration cordis
- IO: Interstitial oedema
- OF: Other findings

Extramedullary erythropoiesis and granulopoiesis:

- R: Retarded extramedullary erythropoiesis or granulopoiesis

Immature erythroctes in the peripheral blood:

- Y: Yes
- No: No immature erythrocytes seen in peripheral blood

Bone marrow colonisation:

- P: Poorly colonised
- N: Normal for the age

Cerebrum status:

- Nv: Neuropil vacuolation
- Nd: Neuronal vacuolar degeneration
- I: Immature cerebrum
- An: Anaemia

Spinal cord status:

- Nv: Neuropil vacuolation
- Nd: Neuronal vacuolar degeneration

Bacterial findings:

- S: Septicaemia
- 2: Secondary infection
- L: Local infection
- NR: Bacteria found not relevant
- No: No bacteria found

ACKNOWLEDGMENTS

To **Prof. Dr. E. F. Kaleta** from the University of Gieβen, for his contribution and interest in the avian medicine, and for guiding and advising me with his experience in this doctoral thesis. I am grateful to him for accepting me as an external doctorand, and for encouraging, guiding and helping me in the avian medicine.

I specially appreciate the instruction, advice and technical help of **Prof. Dr. H. Gerlach**, without which it would not have been possible to finish this project and the doctoral thesis. I would like also to thank her for a comfortable stage in Munich.

Thanks to **Loro Parque Fundación** and its director, **Dr. D. Waugh**, for the financial support and technical help that made possible this study. I appreciate the opportunity of working with the organization and with the biggest parrot collection in the world.

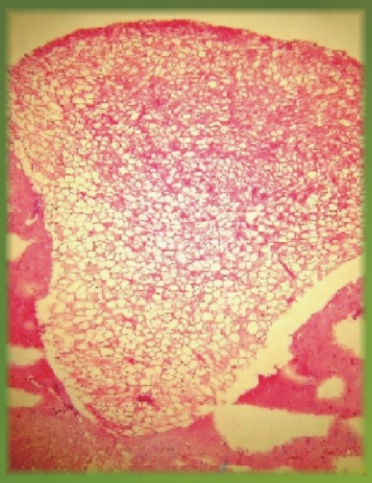
Thank you very much to **Dr. L. G. Crosta** and **Dr. M. Bürkle** for trusting me to carry out this project. Their advice, encouragement and instruction in avian medicine has been fundamental for my professional education. I truly appreciate their educational labour and their nice working team.

Prof. Dr. Dr. Schmahl from the Ludwig–Maximilians University in Munich, thanks for his technical help discussing with his chicken chicks experience the histopathological findings with Prof. Dr. H. Gerlach.

The support, encouragment and financial help of **my family** have always been there whenever necessary and helped me to go on with the project and in my professional life, thank you from the bottom of my heart.

My gratitude to **Cristine Jo Dreisörner** for her patience and help, always with a smile in her face. I truly appreciate the English corrections of **Dr. M. Dune** and **Dr. D. Waugh**. Thanks to **Dr. S. Clubb** for helping me in the bibliographic collection. I am grateful to the laboratory "Labor für Tierpathologie" of **Dr. E. von Bomhard** in Munich for processing the histological samples.

Thanks also to: **Dr. L. Timossi, Matthias Rheinschmidt, Rafa Zamora, Baby Station Working Team, Loro Parque, Núria Viñas, Montse Couselo** and **many other friends.**



édition scientifique
VVB LAUFERSWEILER VERLAG

VVB LAUFERSWEILER VERLAG
STAUFENBERGRING 15
D-35396 GIESSEN

Tel: 0641-5599888 Fax: -5599890
redaktion@doktorverlag.de
www.doktorverlag.de

ISBN 3-8359-5095-9

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