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Proceedings of the 2014 National Toxicology Program Satellite Symposium

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Abstract

The 2014 annual National Toxicology Program (NTP) Satellite Symposium, entitled “Pathology Potpourri” was held in Washington DC, in advance of the Society of Toxicologic Pathology’s 33rd annual meeting. The goal of this annual NTP Symposium is to present current diagnostic pathology or nomenclature issues to the toxicologic pathology community. This article presents summaries of the speakers’ presentations, including diagnostic or nomenclature issues that were presented, along with select images that were used for audience voting and discussion. Some lesions and topics covered during the symposium included a pulmonary mucinous adenocarcinoma in a male B6C3F1 mouse; plexiform vasculopathy in Wistar Han rats; staging of the estrous cycle in rats and mice; peri-islet fibrosis, hemorrhage, lobular atrophy and inflammation in male Sprague Dawley rats; retinal dysplasia in Wistar Han rats and B6C3F1 mice; multicentric lymphoma with intravascular microemboli and tumor lysis syndrome, and two cases of myopathy and vascular anomaly in Tg.rasH2 mice; benign thymomas in Wistar Han rats; angiomatous lesions in the mesenteric lymph nodes of Wistar Han rats; an unusual foveal lesion in a cynomolgous monkey; and finally a series of nomenclatures challenges from the endocrine International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) organ working group (OWG).

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Keywords

NTP Satellite Symposium; mucinous adenoma; plexiform vasculopathy; vaginal cytology; chronic-active pancreatitis; retinal dysplasia; thymoma; mesenteric lymph node angiomatous lesion; fovea cysts; endocrine nomenclature; acute tumor lysis syndrome

INTRODUCTION

The NTP Satellite Symposium is a one-day meeting that is traditionally held in conjunction with the annual Society of Toxicologic Pathology (STP) meeting (Bach et al. 2010; Adams et al. 2011; Elmore et al. 2012; Elmore et al. 2013). Attendance at the symposium has steadily increased since the first meeting in 2000 and this year there were 247 registered participants. The objective of this annual symposium is to provide continuing education on interpreting histopathology slides. This includes the presentation and discussion of diagnostically difficult, interesting, or rare lesions, or challenging nomenclature issues. The session is interactive in that each speaker presents images for audience voting via wireless keypads. Once the votes are tallied the results are displayed on the screen for all to view. The speaker generally provides his or her preferred diagnosis for comparison with some additional background information, after which lively and constructive discussion ensues.

The theme for the 2014 Symposium was “Pathology Potpourri” which allowed for a variety of topics to be presented. Case presentations covered a variety of species that included rat, mouse, and monkey, and presented tissues and/or organ systems that included pulmonary, vascular, pancreas, ocular, thymus, and mesenteric lymph node. Several of the lesions were from the Wistar Han rat, which the NTP used briefly before switching from the F344/N rat to the Sprague Dawley rat (King-Herbert 2010). This new rat strain provided several lesions that had not previously been seen or diagnosed by the NTP pathology group.

Also presented was a series of vaginal smears from an NTP clinical pathologist, with challenges to diagnose the correct estrous stages. This presentation is a prelude to an invited review that will be published in 2015. And finally, one speaker presented diagnostic challenges from the adrenal, thyroid and pituitary glands that the INHAND Endocrine OWG had been considering. This article provides synopses of all presentations including the diagnostic or nomenclature issues, a selection of images presented for voting and discussion, voting choices, voting results, and major discussion points.

AN UNUSUAL LUNG LESION IN A MOUSE

The first presentation, given by Dr. Margarita M. Gruebbel (Experimental Pathology Laboratories, Inc. Research Triangle Park, NC, USA), was an unusual lung lesion in an untreated control male B6C3F1 mouse from an NTP 2-year dosed-feed study. The animal was sacrificed on Study Day 647 due to moribundity from an extraneous cause (hepatocellular carcinoma).

At necropsy, the right diaphragmatic lung lobe exhibited a single, $6.0 \times 3.0 \times 2.0$ mm, firm, irregularly shaped, pale mass. The lung lobe was fixed in 10% neutral buffered formalin,

embedded in paraffin, and microtomed onto glass slides. Sections on glass slides were stained with hematoxylin and eosin (H&E), with Alcian blue (AB) at pH 1.0 and pH 2.5, or with periodic acid Schiff reaction (PAS) with and without diastase. Formalin-fixed, paraffin-embedded lung sections also underwent immunohistochemical staining for club (Clara) cell-specific protein (CCSP) with rabbit anti-CCSP polyclonal antibody (Seven Hills Bioreagents, Cincinnati, OH) as the primary antibody, and for surfactant protein C (SPC) with rabbit anti-prosurfactant protein C antibody (Millipore, Billerica, MA) as the primary antibody.

Light microscopic images (stained with H&E, AB, PAS, or for CCSP or SPC) of the lung mass (Figures 1A–F) were then presented, with the following voting choices and results: lung-bronchiolar epithelium, metaplasia, mucous (9%); lung – alveolar epithelium, metaplasia, mucous (20%); lung – acinar carcinoma (mucinous subtype) (6%); lung – alveolar/bronchiolar adenoma, mucinous (38%); lung- alveolar/bronchiolar carcinoma, mucinous (9%); lung–Bmucinous adenoma (13%); and lung–mucinous adenocarcinoma (7%). Thus, the majority consensus (51%) was that the lung finding was a benign neoplasm with mucinous phenotype rather than a metaplastic change. The speaker agreed with this diagnosis. The plurality of votes were for a lung, *alveolar/bronchiolar adenoma, mucinous* (38%).

Light microscopic examination revealed a discrete, nonencapsulated, oval mass which was located in the alveolar parenchyma and slightly elevated the peripheral edge of the affected lobe (Figure 1A). The predominant histologic pattern consisted of acinar-like structures (Figure 1B) supported by a moderately abundant fibrous stroma containing a mild inflammatory infiltrate (small mononuclear cells, macrophages, and neutrophils). Scattered solid nests of similar cells were uncommon. A few apparently entrapped, large bronchioles were also scattered throughout the mass, and in some sections appeared to branch from a bronchus which coursed adjacent to the innermost (parenchymal) border of the mass (Figure 1A).

The acinar structures were lined by homogeneous, closely packed, tall, plump, columnar cells whose long axis was perpendicular to the acinar basement membrane (Figure 1B). The cells were generally aligned in single layers, with only occasional, focal “piling up”. The cell apical surfaces were smooth and often slightly rounded, but lacked cilia. Nuclei were generally small, uniform (oval to round) and often aligned in single basal rows, though larger, more vesicular, and centralized nuclei also occurred. Mitotic figures were very rare. Acinar lumens were generally clear, though a few contained clumps of inflammatory cells and/or a few sloughed lining cells.

The abundant cytoplasm of acinar and solid-nest cells was pale pinkish-blue in H&E sections (Figure 1B). The cytoplasm stained intensely positive (bright magenta) with the PAS reaction (with and without diastase)(Figure 1C). The cytoplasm did not stain with AB at pH 1.0, but was strongly positive (deep turquoise) with the AB stain at pH 2.5 (Figure 1C). These results are consistent with the properties of cytoplasmic mucin. The great majority of acinar and solidnest cells were negative for CCSP antibody staining, though a

few scattered single cells did exhibit variable CCSP positive staining (Figure 1D). Acinar and solid-nest cells were negative for SPC staining (Figure 1E).

The inner border of the mass caused little if any compression of the alveolar parenchyma, and tended to blend diffusely with adjacent alveolar septa. In some areas, low columnar cells with pinkish-blue cytoplasm appeared to extend from the mass and along existing adjacent alveolar septa (lepidic growth pattern) (Figure 1F).

The occasional dilated larger bronchioles within the mass were lined primarily by cuboidal to low columnar, usually nonciliated cells with pale pinkish cytoplasm and oval to round nuclei; these bronchiolar cells were PAS-negative and AB-negative, mainly CCSP-positive, and SPC-negative. The bronchiolar cells were sometimes arrayed in a single layer, but were occasionally crowded in multiple layers. Where these larger bronchioles debouched directly into the acinar structures, there was a strikingly abrupt transition from the PAS- and AB-negative bronchiolar epithelium to the PAS- and AB-positive acinar epithelium.

The lining epithelium of the respiratory tract conducting airways consists of several cell types, the proportions of which vary with location and species. In normal human lungs, mucous (goblet) cells are abundant only in the proximal conducting airways (bronchi and larger bronchioles), while club (Clara) cells (Winkelmann and Noack 2010) are found primarily in the distal airways, constituting approximately 11% and 22% of the lining cells of terminal and respiratory bronchioles, respectively (Boers et al. 1999; Williams et al. 2006). In mouse lungs, mucous (goblet) cells are quite scanty and normally restricted to the bronchi, but pulmonary club (Clara) cells are very abundant, constituting approximately 50–70% of lining cells at all conducting airway levels (bronchi to terminal bronchioles), with the remainder being mainly ciliated cells; (Boucherat et al. 2013; Curran and Cohn 2010; Evans et al. 2004; Pack et al. 1981; Toskala et al. 2005; Williams et al. 2006). These numerous constituent club (Clara) cells produce the low, physiological levels of mucins normally present in mouse conducting airways (Zhu et al. 2008).

Nonneoplastic increases in conducting airway mucous (goblet) cells occur in many human lung diseases including asthma, cystic fibrosis, and chronic obstructive pulmonary disease (COPD) (Curran and Cohn 2010; Williams et al. 2006), and can also occur due to other causes such as smoking (Seatta et al. 2000). In mice, mucous cell increases can be induced by various experimental methods, including the ovalbumin-induced allergic asthma model (Reader et al. 2003); genetic modifications (Mall et al. 2004) and exposure to various noxious agents such as bacterial toxins (Caldwell et al. 2009), allergens and particulates (Takahashi et al. 2010), viruses (Walter et al. 2002), and certain carcinogens (Rehm and Kelloff 1991).

In many studies, these increases in both species have been referred to by various descriptive diagnoses such as “mucous cell” and/or “goblet cell” “metaplasia” and/or “hyperplasia”, but in many cases, no evidence is provided to support the pathogenic mechanism(s) and/or presumed cell of origin implied by such descriptive terminology.

However, some studies conducted in mice do provide evidence that, at least in this species, the predominant mechanism for airway mucous cell increases is in fact transdifferentiation

(“metaplasia”) of constituent club (Clara) cells to a more typical goblet-cell phenotype demonstrating increased mucin secretion, with little or no proliferation or “hyperplasia” (Boucherat et al. 2013; Evans et al. 2004).

In both mice and humans, nonneoplastic pulmonary mucous (goblet) cell increases are almost always confined to the conducting airways (Evans et al. 2004; Reader et al. 2003; Walter et al. 2002), with purely alveolar occurrence very rarely reported in humans (Nazarian et al. 1989) or mice (Renne et al. 2009).

Most spontaneous and induced epithelial lung neoplasms in mice are localized in the alveolar parenchyma and distal bronchiolar regions. In many cases, the cell(s) of origin (Type II alveolar cell, club [Clara] cell, and/or various putative stem cells) remains undetermined and/or controversial (Meuwissen and Berns 2005; Renne et al. 2009). Therefore murine adenomas/adenocarcinomas are often assigned, by default, the modifier “bronchioloalveolar” (Renne et al. 2009), though not all authors recommend this common practice (Nikitin et al. 2004). These neoplasms can exhibit a range of histologic growth patterns (e.g., lepidic, solid, papillary, acinar, or mixed) (Meuwissen and Berns 2005; Nikitin et al. 2004; Renne et al. 2009).

In humans, mucinous adenocarcinoma subtypes with variable degrees of invasiveness can occur in the lung (Travis et al. 2013). In mice, predominantly mucinous differentiation has been documented in some experimentally induced mouse epithelial lung neoplasms (Maeda et al. 2012; Rehm and Kelloff 1991), but it is rarely seen in spontaneously occurring neoplasms (Renne et al. 2009). Some authors consider that these murine mucinous-type neoplasms arise from club (Clara) cells (Renne et al. 2009).

The present case demonstrated a predominantly acinar morphology. The mass was located in the alveolar parenchyma, but the peripheral lepidic growth pattern and the continuity of acinar structures and entrapped bronchioles were suggestive of extension into the alveolar parenchyma from an origin in conducting airway epithelium, a tissue that in mice is replete with club (Clara) cells but essentially lacks mucous (goblet) cells. The great majority of neoplastic cells were negative for CCSP, an immunohistochemical marker for club (Clara) cells), though a few CCSP-positive cells were occasionally present in acini. The neoplastic cells were uniformly negative for SPC, an immunohistochemical marker for Type II alveolar cells. Virtually all neoplastic cells were strongly PAS- and AB-positive, providing clear evidence of mucinous production.

After discussion, a revote was taken and the top two votes were lung, *mucinous adenoma* (44%) and lung, *alveolar/bronchiolar adenoma, mucinous* (21%).

Based on these results, the case presented here was considered most consistent with a pulmonary mucinous adenoma (with acinar morphology). The occurrence of this neoplasm in an untreated control mouse indicated that it was a spontaneous finding. While the histogenesis was not determined, it is tempting to consider as a possibility the neoplastic transformation of club (Clara) cells, with subsequent altered gene expression resulting in a mucous-cell phenotype.

PERPLEXING VASCULAR LESIONS IN WISTAR HAN RATS

Dr. Jessica S. Hoane (Charles River Laboratories Pathology Associates, Durham, NC) presented a rarely reported vascular change present in a few Wistar Han rats from an as yet unpublished NTP chronic particulate inhalation toxicity and carcinogenicity bioassay. The other pathologists involved in the review of this study included the NTP pathologist (Dr. Gordon Flake, NTP, NIEHS, RTP, NC), the study pathologist (Dr. Laurie Staska, Battelle Northwest, currently at WIL Research, Hillsborough, NC), and the quality assurance pathologist (Dr. E. Terence Adams, formerly of EPL, Inc., Research Triangle Park, NC). In addition, Dr. Abraham Nyska (Independent consultant, Timrat, Israel) provided pivotal insight and resources after evaluating digital photomicrographs of the vascular lesions.

Male and female Wistar Han (CrI:WI[Han]) rats were exposed to the test material via inhalation for 6 hours daily, 5 days/week, excluding holidays, for up to 105 weeks. Ten animals from all male and female dose groups were randomly selected for an interim sacrifice after one year on study. An increase in vascular lesions predominantly diagnosed as “blood vessel, chronic active inflammation” and “blood vessel, necrosis” was seen in the mid and high dose males and at all doses in the females on the 105-week study, but no vascular changes were noted at the one year interim time point. These lesions were not appreciated grossly, and though not designated for collection, the vessels were routinely present in the connective tissue adjacent to the thymus and mesenteric lymph node. As the reviewing pathologist, Dr. Hoane was specifically requested to look at mesenteric vessels for inflammation and necrosis, but found vascular lesions in other tissues as well.

Low and high magnification images from three separate high-dose females were presented and a single vote was taken. The first two examples were from large mediastinal vessels, and the predominant feature was the formation of numerous variably sized and shaped endothelial-lined vascular spaces expanding the tunica media of the vessel (Figures 2A and 2B). Little inflammation was associated with these lesions. The third example was from a mesenteric vessel, determined most likely to be a vein, and was characterized by tortuous, incomplete endothelial-lined vascular spaces expanding and dissecting through the tunica media (Figures 2C and 2D). The endothelial cells lining the spaces in all three examples were relatively flattened and quiescent with no evidence of pleomorphism or mitotic figures. The voting choices made available to the audience were (1) polyarteritis, (2) hemangioma, (3) hemangiosarcoma, (4) endothelial dysplasia, (5) endothelial hyperplasia, (6) plexiform vasculopathy, (7) dilatation of Vasa Vasorum and (8) other. The majority of attendees selected *plexiform vasculopathy* (52%). The other diagnoses received from 3 to 19% of the votes: polyarteritis (3%), hemangioma (5%), hemangiosarcoma (6%), endothelial dysplasia (7%), endothelial hyperplasia (5%), dilatation of vasa vasorum (19%) and other (3%).

Plexiform vasculopathy, described by Westwood et al., is the presence of numerous distended vascular channels within the media and adventitia, sometimes with prominent cavernous and tortuous channels (Westwood et al. 1990). This finding was seen in the mesenteric veins of mid and high dose males and at all doses in females in a six month study of a phosphodiesterase (PDE) inhibitor with both inotropic and vasodilator properties (Westwood et al. 1990).

Most of the vascular lesions seen in this study were characterized by chronic active inflammatory infiltrates both within the vessel walls, sometimes expanding out in the adjacent adventitia. The lesions resemble the spontaneous lesion of polyarteritis. There was also occasional necrosis of the vascular wall, typically the tunica media, and often subintimal expansion by homogenous eosinophilic proteinaceous material (fibrin; fibrinoid necrosis), both of which were combined under the diagnosis of “necrosis.”

Though formation of small capillary-type vessels within these lesions occasionally occurred, only the three females shown in the presentation had extensive intra-mural vascular channel formation. Two females on this study were affected in large mediastinal vessels, and it was difficult to tell whether the vessels were arteries or veins due to the extent of the vascular wall distortion. The third female was affected in the mesenteric vessel (likely vein) with an appearance very similar to the previously published representative images of plexiform vasculopathy (Westwood et al. 1990).

As this is a rarely reported lesion, the pathogenesis of this particular vascular change is unclear. However, as vascular changes consisting of inflammation, serum exudation and necrosis are spontaneous changes in rats and have also been shown to occur at greater incidence in response to numerous vasoactive substances (“drug induced vascular injury [DIVI]”), this is a well-studied phenomenon (Morton et al. 2014). The general and molecular pathogenesis of the more well known vascular lesions were presented during the discussion portion of the presentation.

Early studies of vascular injury relied heavily on electron microscopy, and there was debate as to whether the initial injurious change was within the tunica media or endothelium or if both occurred simultaneously (Goldby and Beilin 1972; Takebayashi 1970; Thorball and Olsen 1974; Todd and Friedman 1972). There was general agreement within the scientific community that the lesions most likely occurred due to alternating segments of arterial dilation and constriction, predominantly within the dilated regions, and the regions of varying vascular diameter could be appreciated grossly (Giese 1964; Goldby and Beilin 1972; Kerns et al. 1989; Thorball and Olsen 1974). It was thought that vascular injury may result when a critical wall tension is exceeded (Nemes et al. 1980).

One early change seen, within 15 minutes of administration of L-norepinephrine, was an increase in the number of cell-to-cell hernias in which a portion of the cytoplasm of a smooth muscle cell within the tunica media invaginated into an adjacent smooth muscle cell (Joris and Majno 1981), and this change is evident on light microscopy as vacuolation (Figure 2E, arrows). Necrosis and hemorrhage of the tunica media are thought to be early changes in vascular injury (Figures 2E and 2H, white arrowheads) (Takebayashi 1970; Todd and Friedman 1972; Yuhas et al. 1985), and are likely sequelae to the initial vacuolation. The subintimal accumulation of serum protein also occurs rapidly at sites of vascular injury (Goldby and Beilin 1972; Takebayashi 1970), and was present to varying extents in many animals on this current study (Figures 2F and 2G, arrows). Varying extent of adventitial inflammation was also common (Figures 2E–H, arrowheads).

More recent studies of DIVI have taken a more molecular approach. Many studies have focused on two well-known vascular-lesion inducing agents, fenoldopam mesylate, a dopaminergic vasodilator, and PDE inhibitors. Nitrate stress was found to be a prominent feature of PDE inhibitors (Slim et al. 2003; Weaver et al. 2010; Zhang et al. 2008), and more recent studies showed that nitric oxide (NO) was involved in vascular injury both due to PDE inhibitors (Sheth et al. 2011) and fenoldopam mesylate (Brott et al. 2012). More specifically, alterations in the phosphorylation of endothelial nitric oxide synthase (eNOS) occurred after administration of several drugs known to cause DIVI, (Tobin et al. 2014). The change in NO within the vessel wall likely results in increased oxygen free radical formation and explains the quick inflammatory response often seen at the sites of vascular injury.

Other recent findings in molecular studies of fenoldopam mesylate-induced vascular injury include the discovery of decreased caveolin-1 expression and loss or decreased expression of caveolin-1 regulated proteins at the site of the injury (Brott et al. 2012). Caveolin-1 is essential for regulation of tight and gap junction proteins, which maintain vascular wall integrity (Brott et al. 2012). It is likely that these alterations in the endothelial cell junctions results in the subintimal deposition of plasma proteins within the vascular wall at injured sites.

Though the animals on the current study were not administered an exogenous vasoactive compound, significant changes in the lungs, including proteinosis, fibrosis, chronic active inflammation and epithelial hyperplasia, likely had significant effect on gas exchange in exposed animals. Hypoxia was evident grossly and cyanosis and abnormal breathing were common clinical observations and were more prevalent in females. In addition, the incidence of proliferative lesions of the adrenal medulla, including hyperplasia and benign pheochromocytoma, were also increased in treated males and females. This likely resulted in an increased secretion of endogenous vasoactive compounds within the body, trying to maintain some degree of homeostasis despite the difficulties with oxygenation.

Though plexiform vasculopathy has not been diagnosed in prior NTP studies, systemic vascular injury was present in male B6C3F1 mice on a number of particulate inhalation chronic studies conducted by the NTP (Moyer et al. 2002). Vascular changes in these studies were noted in main coronary arteries of the heart, other arteries within the heart and/or arteries within the kidney. Vascular lesions were not seen as an exposure-related effect on the companion B6C3F1 mouse study to the current study; however, the lung changes seen within the mice varied considerably from the rats and there was no significant increase in adrenal medullary proliferative lesions (unpublished data).

Vascular injury was not reported in rats in the prior particulate inhalation studies previously conducted by the NTP; however, these studies were done in F344/N rats. Mesenteric periarteritis was seen, though, in F344/N rats after administration of theophylline for 16-days, 13-weeks and 2-years (Collins et al. 1988; Nyska et al. 1998). The location of the lesions in the current study is unusual, as mediastinal vessels have not been reported as a site for vascular injury. It was thought that most of the vascular changes were occurring within medium to large arteries, but in some instances the extent of the change precluded proper identification of the vessel type.

One audience member questioned the statement from the take home points that vascular injury occurred in an unusual location and “progressed” in a small number of animals to plexiform vasculopathy. The presenter agreed that a progression from the typical inflammatory lesions seen with vascular injury to plexiform vasculopathy is unlikely due to the lack of findings typical of post inflammatory repair. It is unclear at this time why a small number of animals on this study deviated from what is typically seen with vascular injury in rats (e.g. inflammation and necrosis), and instead had an unusual, rarely reported creation of numerous vascular spaces within the adventitia and/or media of the affected vessels.

THE ABCS OF PEMD – VAGINAL CYTOLOGY OF THE RAT AND MOUSE

Dr. Michelle Cora (NIEHS, NTP, Research Triangle Park, NC, USA) presented basic criteria for the classification of the four classic stages of the estrous cycle in mice and rats (Proestrus, Estrus, Metestrus and Diestrus: PEMD) as is seen on dry-fixed slides stained with Romanosky-type stains. After a brief introduction, an initial quiz was presented whereby two different photomicrographs of the estrous cycle were voted on by the audience. One slide showed a photomicrograph of diestrus and the other of proestrus. The majority of the audience voted correctly on both stages with 46% voting for *diestrus* and 44% voting for *proestrus*.

There are four cell types that are seen in the stages of the estrous cycle—neutrophils, small nucleated epithelial cells, large nucleated epithelial cells and anucleated keratinized epithelial cells. Neutrophils (leukocytes, polymorphonuclear cells) are small and round with a multilobulated nucleus (Figure 3A). They may shrink in the process of preparing the slide, precluding visualization of the nuclei. Small nucleated epithelial cells are round to oval with round nuclei; they may stain deeply basophilic (Figure 3B). Large nucleated epithelial cells possess a lower nuclear:cytoplasmic ratio (more cytoplasm) compared to small epithelial cells (Figures 3A & 3C). They may have irregular, jagged or angular borders with a nucleus that may be intact, degenerate or pyknotic. Anucleated epithelial cells are aged and keratinized with jagged or angular borders and possess remnants of a nucleus or no nucleus; “ghost nuclei” may be present (Figures 3C & 3D).

The stages are defined by the presence, absence or proportion of the four above described cell types (Long and Evans 1922; Allen 1922; Goldman et al. 2007; Hubscher et al. 2005; Byers et al. 2012). The estrous cycle of both rats and mice has an average duration of 4–5 days. Proestrus has an average duration of 12–18 hours and is characterized by the presence of high numbers of small nucleated epithelial cells found individually and in clusters, strands or sheets (Figures 3B & 3E). A few neutrophils may be seen in early proestrus. Lower numbers of large and anucleated epithelial cells may also be seen; however, the presence of lower numbers of neutrophils or large and anucleated epithelial cells does not preclude the diagnosis of proestrus when the predominant cell type is the small nucleated epithelial cells.

Estrus has an average duration of 24–48 hours in both rats and mice and is characterized by high numbers of anucleated epithelial cells (Figures 3D & 3F). Bacteria can be seen adhered to the anucleated cells (Figure 3C). Rare neutrophils may be seen in late estrus as the rodent

is transitioning into metestrus. In both rats and mice a few nucleated epithelial cells may be noted throughout estrus; however, rats (unlike mice) display high numbers of small to large nucleated epithelial cells interspersed among the anucleated epithelial cells in late estrus (Figures 3C & 3G). These cells are spindle, oval or round in shape and usually have uneven borders; the large nucleated epithelial cells of late estrus are sometimes referred to as “pavement” cells (Young et al. 1941). Due to the short duration of this late estrus “stage” in rats, it may not always be appreciated.

Metestrus is a short stage in rats, lasting only an average of 8 hours, while in the mouse it may last up to 24 hours. In the mouse this stage is characterized by the combination of anucleated epithelial cells and neutrophils with possibly low numbers of nucleated epithelial cells, while in the rat, metestrus is characterized by anucleated epithelial cells, small and large nucleated epithelial cells (of late estrus) and neutrophils. In both species, neutrophils are intermixed with the anucleated cells (and nucleated cells in the case of rats) in early metestrus (Figures 3H & 3I) with the peak of metestrus distinguished by its higher cellularity due to a flood of neutrophils that greatly outnumber the epithelial cells (Figure 3J). The neutrophils become less in number as the rodent transitions to diestrus.

Diestrus is the longest stage of the estrous cycle with an average length of 48–72 hours. This stage is characterized by the combination of neutrophils, large and small epithelial cells, and, to a lesser extent, anucleated epithelial cells (Figures 3A & 3K). Diestrus also has a decrease in cellularity as compared to mid- and late metestrus. Neutrophil numbers vary, but are generally higher in number than the epithelial cells, with some smears being exclusively neutrophilic.

Because the estrous cycle is a steadily changing process, nuances exist in dividing the cycle into four stages. Proper training and experience, as well as agreement on the criteria for the identification of the stages (within any particular laboratory) are required for accurate and consistent vaginal cytology reads. This is particularly important in the event of transitional stages when the evaluation can be more subjective despite established objective criteria.

At the end of the presentation four photomicrographs depicting the four stages of the estrous cycle were shown in random order to the audience for voting. In all four cases, identification of the stages improved overall with 63%, 69%, 78%, 77% of the audience voting correctly for *proestrus*, *diestrus*, *estrus* and *metestrus*, respectively.

A PUZZLING PANCREATIC PROBLEM

Dr. Rachel Peters (Takeda Pharmaceuticals International, Co., Cambridge MA USA) presented a case that involved pancreatic lesions from a male Sprague Dawley rat. This rat was part of mid-dose group in a 28 day GLP (good laboratory practice) toxicity study and was treated via oral gavage once a day for 28 consecutive days. All rats were 12 weeks old at the initiation of study and the test article was dosed in 10% hydroxypropyl- β -cyclodextrin vehicle. Audience members were instructed to vote on the best diagnosis that captured the morphologic changes and possible pathogenesis of the lesion. The voting choices and results were: acinar atrophy (3%), acute pancreatitis (1%), chronic-active pancreatitis (27%),

spontaneous islet hemorrhage and fibrosis of the SD rat (9%), *peri-islet fibrosis*, *hemorrhage*, *lobular atrophy and inflammation* (60%), and other (0%).

The histopathologic features of the lesion included locally extensive areas of fibroplasia and mononuclear to mixed inflammation containing atrophic acini and entrapped islets; inflammatory cells and fibroplasia along interlobular and peri-pancreatic tissue; as well as inflammation and hemorrhage in islets (Figures 4A–D). The diagnosis rendered was chronic-active pancreatitis but other diagnoses were sought that may better capture the features of this lesion.

At this point, study data were presented. This finding spanned a range of severities and was present at all dose levels but was only seen in males and largely within the mid-dose group (6/10 mid-dose animals). Two of these animals were identified with islet-centered hemorrhage and inflammation. The high dose was not tolerated with early mortalities and subsequent dose reduction: pancreatic inflammation was seen in one high dose male at final necropsy but not in the early mortalities. This finding was not present in recovery rats or in female rats with the caveat that there were only low numbers of recovery animals since the recovery group was set only for the high dose group. Given the acinar and islet involvement present in the findings, three possible pathogenic mechanisms for this finding were presented: 1) exocrine injury with bystander endocrine injury, 2) islet injury with bystander exocrine injury and 3) interface pancreatic injury. Brief summaries of the histopathologic features of these three mechanisms were presented with comparison to the changes seen in this particular case.

Exocrine pancreatic injury induced in the rat by non-invasive methods (administration of cerulein, cyanohydroxybutene and large amounts of select amino acids such as L-arginine, L-ornithine) is characterized by inflammation, lobular atrophy, acinar cell death, acinar cell vacuolar degeneration, and/or replacement of exocrine tissue by fibrosis and adipose tissue (Cattley et al. 2013, Kui et al. 2014, Usborne et al. 2014). The patchy acinar involvement with islet changes seemed to rule out primary exocrine injury in this case.

Endocrine pancreatic injury in the rat can be induced chemically (streptozotocin, cyclosporin A, alloxan) or can be related to strain and/or dietary intervention (BB Wistar rat, Zucker diabetic fatty rat, Goto-Kakizaki rat). These models demonstrate islet specific degeneration, necrosis, inflammation and/or fibrosis with a subset having varying degrees of concurrent exocrine involvement (Chandra et al. 2013, Jones et al. 2013). The extensive acinar involvement in this case did not seem compatible with the published models of endocrine injury.

Interface pancreatic injury is a relatively new term proposed to describe injury at the exocrine and endocrine interface. The underlying cause is proposed to be from vascular injury to the capillaries at the endocrine-exocrine interface with subsequent peri-islet hemorrhage, fibrin deposition and/or vasodilation. In the chronic stages, there is inflammation, islet fibrosis and/or degeneration/necrosis or atrophy of surrounding acinar tissue. This was reported as a test-article related change affecting both male and female rats and there is evidence to suggest that this is a species-specific finding, as subsequent studies

in dogs and primates did not have this finding (Brenneman et al. 2014). The histologic features of this entity seemed to match what was seen in this case except that these changes, although test-article related, were seen only in males.

Given the gender disparity, test-article exaggerated background changes were considered. These findings share some features with background acinar atrophy but, in this case, the islets were not spared, the inflammatory response was more severe, and the controls did not demonstrate a background change of acinar atrophy (McInnes 2012a). Islet hemorrhage and fibrosis has been reported as a background finding in older male SD rats that is similar morphologically to the peri-islet findings in this case (Imaoka et al. 2007). However, the rats were young (16 weeks at the termination of study), there was extensive acinar involvement, and the controls did not have this change as a background finding.

A summary table of key comparison features of this finding against the three major types of pancreatic injury, as well as reported background findings, was presented (Table 1). Note that all of the findings can have inflammatory cell infiltrates/inflammation with varying degrees of acinar involvement as a feature. During the discussion, it was raised that the proposed interface injury, background changes, islet and peri-islet changes and pancreatic islet injury may share pathogenic mechanisms given their similar histologic appearances and that a test article, such as in this case, may exacerbate background findings. In addition, it was also raised that the interface injury classification was relatively newly characterized and may be subject to revision in the future. In terms of nomenclature, Charlotte Keenan from INHAND GESC discussed categorizing the changes by process (inflammation/hemorrhage) and then using different locators (islet/acini) to convey the nature of the findings in this case. After discussions, a revote was taken with a split majority vote between *chronic-active pancreatitis* (45%) and *peri-islet fibrosis, hemorrhage, lobular atrophy and inflammation* (45%).

EYE-CATCHING FINDINGS IN THE RODENT RETINA

Dr. Kathleen A. Szabo (Charles River Laboratories, Durham, NC, USA) presented cases of retinal dysplasia in rats and mice as a terminology dilemma. Contributions to this talk were made by Drs. Gordon Flake, Margarita Gruebbel, and Deepa Rao. Two sets of eye cases (one rat and one mouse) were presented for voting. The majority of images were from a 90-day subchronic inhalation toxicity study that was conducted in male and female Wistar Han (CrI:WI[Han]) rats and B6C3F1 mice. The potential diagnostic choices for both cases included retinal dysplasia, sectioning artifact, retinal degeneration, fixed fold formation of the retina, developmental anomaly of the retina, and other. For the set of cases representing the rat (Figure 5A), the majority (42%) of the audience favored *retinal dysplasia* followed closely by developmental anomaly (32%), and then fixed fold formation (16%), sectioning artifact (6%), and degeneration (4%). In contrast, 38% of the audience voted for fixed fold formation, followed by developmental anomaly (25%), dysplasia (18%), other (11%), sectioning artifact (7%), and degeneration (1%), for the cases representing the mouse (Figure 5B). Dr. Szabo briefly discussed each of these possibilities: 1) sectioning artifact would lack accompanying histopathology changes of the retina (Dubielzig et al. 2010), 2) retinal degeneration is typically recognized in older rats and mice as a decreased thickness

of the retina involving the rods, cones, and outer nuclear layer, primarily peripherally, but can progress centrally and include additional layers (Geiss and Yoshitomi 1999; Yoshitomi and Boorman 1990), 3) fixed fold formation has been described in humans as occurring post-operatively after retinal detachment surgery (Behar-Cohen et al. 2000; Heimann and Bopp 2011; Kokolakis et al., 1981), likewise Dubielzig et al. (2010, p. 353) commented that in animals, folds can be recognized after “retinal re-attachment” post-injury, and 4) Dr. Szabo’s viewpoint was that developmental anomaly (Smith et al. 2002) was a good alternative term to dysplasia. The conclusion of this voting discussion was that the preferred term for this finding in both the rats and mice was retinal dysplasia.

A review of retinal neurogenesis was next presented. The approximate dates of retinal development in the mouse are from embryonic day 11 to postnatal day 11 (Zheng et al. 2010; Wallace 2011). A common progenitor cell is the basis of development for both the neuronal cells (ganglion cells, horizontal cells, rods, cones, amacrine cells, bipolar cells) and Müller glial cells (Zheng et al. 2010). Fibroblast growth factor is reported as an initiator of neurogenesis in fish, chickens, and also in mice (Centanin and Wittbrodt 2014), and various signaling pathways and transcription factors are part of the neurogenesis process (Centanin and Wittbrodt 2014; Wallace 2011; Zheng et al. 2010). The developmental maturation is orderly, initially with ganglion cells in the embryonic stage and lastly with Müller cells, primarily postnatal; the remaining neuronal cell types are generated between these two (Centanin and Wittbrodt 2014; Wallace 2011; Zheng et al. 2010). Retinal neurogenesis begins centrally, moves peripherally (Wallace 2011), and appears to be conserved in vertebrates (Centanin and Wittbrodt 2014; Wallace 2011). Around postnatal day 7, cellular proliferation of the retina ends in mice, and several changes occur until postnatal day 21 (Wallace 2011), which result in the typical layered morphology of the mature retina (Zheng et al. 2010). This brief discussion was followed with a photomicrograph of a murine embryonic eye (Figure 5C) to demonstrate the process of normal retinal development. Afterwards, photomicrographs at low and high magnifications of the fully developed (layered) mature rodent retina were shown to the audience to demonstrate the contrast with the immature retina.

Dr. Szabo then relayed more information relevant to retinal dysplasia. A finding described as “retinal folds” was first reported in 1943 in Wistar rats (Poulsom and Hayes 1988) and interestingly, an audience member commented that an earlier report from 1933 exists which describes similar findings (Tansley 1933). Retinal dysplasia occurs in both man and animals, is usually subclinical, and the extent/severity can vary although it is usually focal (Dubielzig et al. 2010; Smith et al. 2002). Retinal dysplasia is commonly thought of as a developmental anomaly (Smith et al. 2002), although it can occur as the result of insult, including viral infection of the dam, irradiation, or experimental manipulation (Percy and Danylchuk 1977; Smith et al. 2002). In rats or inbred black mice, it is considered a background, incidental finding (McInnes 2012b; Smith 2002). The diagnostic morphologic features of retinal dysplasia include an altered appearance of the normal ten-layered retina in the mature eye, characterized by folds, rosettes, and disorganization (Dubielzig et al. 2010; Poulsom and Hayes 1988). Dr. Szabo noted that the finding of retinal dysplasia was identified in only a few animals. In addition, she commented that a query of the NTP website revealed thirty-three records in the Toxicology Data Management System (TDMS) of ‘Eye – retina,

dysplasia' in mice and rats from 1982 forward (National Toxicology Program, 2014). These records represented control and treated animals, subchronic (90-day) and chronic (2-year) studies, and different routes of administration. Final discussion was generated by a participant that centered on the preference to use morphologic terminology of retinal rosette or fold instead of retinal dysplasia because the terminology of dysplasia might imply pathogenesis.

LOOKING BEYOND LYMPHOMA

Dr. Bhanu P. Singh (Janssen Research & Development, Spring House, PA) presented two challenging cases from transgenic rasH2 mice. Collaborators on this project included Janssen colleagues Drs. Saskia De Hoog (study sponsor) and Erio Barale (project pathologist) as well as Madhav Parajpe (Bio Reliance) who was the original study pathologist. The six-month Tg.rasH2 mouse study has gained scientific and regulatory acceptance as a potential replacement for the standard 2-year carcinogenicity mouse bioassay (Nambiar and Morton 2013); this model was created by microinjecting the human c-Ha-ras gene into C57BL/6 × BALBc F2 zygotes. Spontaneous pathological changes in Tg.rasH2 mice can be unique and pose challenges in their evaluation.

Dr Singh's first case focused on acute tumor lysis syndrome (ATLS) in a mouse having disseminated lymphoma. A male Tg.ras H2 mouse from a control group in a 26 week transgenic bioassay showed signs of decreased motor activity and labored breathing around 23 weeks. The mouse was sacrificed and at necropsy a moderate enlargement of multiple lymph nodes, liver, kidneys, spleen and thymus was noted. Microscopically, there was disseminated lymphoma in these organs characterized by extensive infiltration of neoplastic lymphoid cells. In addition to disseminated lymphoma, multifocal intravascular clumps (emboli) of elongated homogeneous, acellular and basophilic material were observed in small vessels of the lungs, adrenal glands, kidneys, heart and liver (Figure 6 A–E). These variably sized emboli were often admixed with fibrillary eosinophilic material in some organs. The emboli were most prominent in the pulmonary vessels. A series of images illustrating microemboli in various organs were presented and the audience was asked to vote for their preferred diagnostic term.

The voting choices and results were multicentric lymphoma (14%), intravascular microemboli (4%), tumor lysis syndrome (12%), choices 1 and 2 (18%), choices 1 and 3 (15%), *choices 1, 2 and 3* (37%). The presenter favored the popular diagnosis of audience.

The microscopic features of this lesion were consistent with acute tumor lysis syndrome. The discussion among audience members mainly focused on what term(s) should be used to describe the lesion. In cases of disseminated lymphoma or leukemia in multiple organs, intravascular microemboli might not get the attention of pathologists/toxicologists or can be masked by the massive number of neoplastic cells. There was also discussion about whether this is a strain-specific lesion in mice. People in the audience and the presenter noted that, in their experience, ATLS has been seen in other strains of mice with lymphoma and leukemia and is not limited to transgenic mice.

The characteristic finding in murine tumor lysis syndrome is the presence of widely disseminated microemboli, composed of nuclear and cytoplasmic debris derived from lysed tumor cells (Treuting et al. 2010). It has been proposed that mechanical obstruction of capillary beds by the microemboli can cause clinical signs of respiratory stress and, in severe cases, can be cause of death (Lovelace et al. 2003). It is not clear what triggers the lysis of tumor cells in this case.

Treuting et al (2010) described histologic, immunohistochemical, and electron microscopic analyses of thirty-one ATLS cases from a cohort of 499 mice that were prone to spontaneous lymphoblastic lymphoma. Seventy-three percent of this cohort died with lymphoblastic lymphoma, and 8% of affected mice died with diffuse microthromboemboli consistent with ATLS. Mice with ATLS had a high spontaneous mortality rate (>50%), a large tumor burden with disseminated disease, and evidence of leukemia. Blood vessels in the lung, kidney, and other organs were occluded by microthromboemboli composed of chromatin, cellular debris, fibrin, platelets, entrapped erythrocytes and malignant cells. Based on this data, the authors have suggested that ATLS can occur at high frequency in mice with disseminated lymphoblastic lymphoma and leads to a high rate of spontaneous death resulting from microthromboemboli.

In human medicine, tumor lysis syndrome is a potentially lethal condition and is precipitated by the massive release of intracellular components, such as nucleic acids, potassium and phosphorous, following a rapid and widespread lysis of tumor cells (Firwana et al. 2012). It is highly associated with rapidly proliferating tumors such as acute lymphoblastic leukemia and high-grade non-Hodgkin lymphoma. Initiation of chemotherapy, radiotherapy, or steroid treatment may trigger ATLS, or it may develop spontaneously. Metabolic disturbances (hyperkalemia, hyperphosphatemia, secondary hypocalcemia, hyperuricemia) are thought to be the primary cause of clinical ATLS symptoms, which include renal dysfunction, seizures, and cardiac arrhythmias (Firwana et al. 2012). The microscopic lesions associated with tumor lysis syndrome in humans are largely unknown because of the low rate of mortality of ATLS in humans and the relatively few clinical cases.

In summary, it is critical to recognize spontaneous tumor lysis syndrome in experimental laboratory animals in order to assess the efficacy and/or toxicity of compounds, because early deaths due to this syndrome might wrongly be attributed to a direct test article effect.

The second case was from skeletal muscle (thigh) of a control female Tg.rasH2 mouse that was a terminally sacrificed at week 26 of a carcinogenicity study. There were no abnormal clinical signs or gross changes during necropsy evaluation. Microscopically, there was multifocal to coalescing areas of muscle degeneration characterized by loss of striation, vacuolation, coagulation and hyalinization of myofibers. These degenerative changes were admixed with moderate numbers of mixed inflammatory cells that included neutrophils, lymphocytes, and macrophages (Figures 6 F–H). The regenerative changes were often noted as a prominent feature in the adjacent myofibers. The regenerating fibers showed basophilic cytoplasm and centrally placed long chains of nuclei (nuclear rowing).

A series of images illustrating these changes were presented and the audience was asked to vote for their preferred diagnostic term. The voting choices and results were degeneration (1%), regeneration (0%), inflammation (0%), choices 1 and 2 (0%), choices 1, 2 and 3 (40%), myopathy (56%), and other (3%). The presenter also favored the audience's popular diagnosis of *myopathy*.

Skeletal myopathy has been observed in almost all Tg.rasH2 mice (Tsuchiya et al. 2002; Paranjpe et al. 2013). In this study, the changes were observed in the *femoralis* and *pectoralis* muscles of the transgenic mice. Inflammatory changes in other skeletal muscles or abnormalities of adjacent peripheral nerves were not observed. In addition, heart, tongue and esophagus showed no microscopic changes. Although the severity of myopathy was relatively mild in younger Tg.rasH2 mice (10 weeks) as compared to older (34-week-old) mice, there was usually no difference in the incidence or severity between male and female mice. In non-transgenic littermates, skeletal myopathy was not observed. The mRNA of the human c-Ha-ras gene was detected in femoral muscle from the Tg.rasH2 mice by RT-PCR, suggesting that integration of the c-Ha-ras gene plays a crucial role in the pathogenesis of skeletal myopathy in the rasH2 mice.

It was readily realized by the audience that the three pathological processes (degeneration, inflammation and regeneration) were interrelated. Because 40% of the audience chose separate diagnoses and 56% of the audience chose "myopathy", the discussion focused on whether one should diagnose three different components of this lesion separately ('splitting') or together ('lumping'). When the pathological processes (e.g. degeneration and inflammation) are observed as distinct events (different pathogeneses), splitting is commonly accepted among pathologists. This approach is often suitable for studies of shorter duration (acute or subchronic) especially when the finding is subtle or minimal in severity. Chronic studies generally have a high incidence of spontaneous background findings. The lesions can appear as different processes (e.g. degeneration, inflammation and regeneration) which are closely interrelated (similar pathogenesis). In this case, splitting of the diagnosis does not provide any additional useful information. Furthermore, it might result in artifactual alterations in one or more diagnoses in a group of animals, causing difficulty in the final analysis and interpretation. The lumping approach has also been supported in the soft tissue and integument INHAND document (Greaves et al. 2013). For the diagnostic term "degeneration", myopathy is listed as a synonym and the authors state: "In view of the overlap between degeneration and other cytological changes that can be seen in skeletal muscle some prefer the more general term myopathy to embrace all such alterations including simple vacuolation".

THROWN OFF TRACK BY THE THYMUS

Dr. Jessica S. Hoane (Charles River Laboratories Pathology Associates, Durham, NC) presented a rarely reported vascular change present in an unpublished National Toxicology Program (NTP) chronic particulate inhalation toxicity and carcinogenicity bioassay. In addition to the pathology working group (PWG), the pathologists involved in the review of this study included the NTP pathologist (Dr. Gordon Flake, NTP, NIEHS, RTP, NC), the study pathologist (Dr. Laurie Staska, Battelle Northwest, currently at WIL Research,

Hillsborough, NC), and the quality assurance pathologist (Dr. E. Terence Adams, previously of EPL, RTP, NC). In addition, Dr. James Morrison (Charles River Laboratories Pathology Associates, Durham, NC) reviewed a select number of the thymus lesions present in this study at the request of the NTP pathologist, and Dr. Kyathanahalli Janardhan (ILS/NTP, RTP, NC) and the NIEHS immunohistochemistry laboratory developed and optimized the cytokeratin-18 protocol used for the select thymus cases.

Dr. Hoane had two cases for voting and rats for both cases came from the same study. Male and female Wistar Han (CrI:WI[Han]) rats were exposed to the test material via inhalation for 6 hours daily, 5 days/week, excluding holidays, for up to 105 weeks. Ten animals from all male and female dose groups were randomly selected for an interim sacrifice after one year on study. Proliferative lesions of the thymus were seen with no exposure-related effect across all dose groups in male and female rats on the 105-week study, but none were noted at the one year interim time point.

The first case presented for voting consisted of a series of low- and high-magnification images of an affected thymus from a female rat which was subgrossly characterized by a roundish, unencapsulated mass with smooth margins that had totally effaced the normal thymus architecture (Figure 7A) and was significantly larger (~10×) than an unaffected thymus from another rat on the 105-week study. At higher magnification (Figure 7B) the mass was predominantly composed of small lymphocytes with dark basophilic, densely condensed nuclei and little cytoplasm; however, closer evaluation revealed an underlying population of larger epithelial cells, with large vesicular nuclei and moderate to abundant pale eosinophilic cytoplasm. Mitotic figures in the epithelial cell population were relatively common (2–3/40X field), though not easily visible to the audience in the image shown. Voting choices made available to the audience were normal, benign thymoma, malignant thymoma, malignant lymphoma, lymphoid hyperplasia, mononuclear cell leukemia, and other. The majority of the voting attendees chose either *benign thymoma* (43%) or *malignant lymphoma* (27%). The other diagnoses received from 0 to 20% of the votes: normal (1%), malignant thymoma (20%), lymphoid hyperplasia (6%), mononuclear cell leukemia (3%), and other (0%). The speaker's preferred diagnosis was benign thymoma.

After voting, cytokeratin-18 immunohistochemical images were presented. A control thymus was shown at low- and high-magnification to illustrate the expected population of cytokeratin-18 positive epithelial cells within the thymus (Figure 7C). Low- and high-magnification images of cytokeratin-18 stained sections of case 1 were also presented to provide the symposium attendees evidence of the extent of the epithelial cell population being masked by the numerous non-neoplastic lymphocytes present within the lesion (Figure 7D). Also, a high-magnification image of a malignant lymphoma effacing the thymus of a Harlan Sprague Dawley from a 2-year bioassay was shown side-by-side with the high-magnification image of case 1 to illustrate the difference in appearance. The malignant lymphoma was composed of a sheet of relatively monomorphic, large lymphocytes with numerous mitotic figures with no remaining thymic epithelial cells, and did not resemble the high-magnification appearance of the lymphocytic-rich thymoma.

During the discussion after the presentation, one audience member questioned whether Case 1 should be considered malignant as it was unencapsulated, had effaced/expanded the thymus considerably, and was likely the cause of moribundity or death in the animal, and the attendee also asked what criteria were used to determine malignancy. In general, invasion and/or metastasis to other tissues are required to consider a thymoma to be malignant. It was a good point, considering the size of the lesion, however, despite the lack of an obvious capsule, the smooth margins and lack of systemic metastases indicated to the presenter that the neoplastic cell population had not extended beyond the thymus tissue and would still be considered a “benign” lesion. After the symposium, one audience member indicated that without the cytokeratin-18 staining, he would have been convinced that Case 1 was a malignant lymphoma, and in fact was surprised at the number of epithelial cells present within the tumor.

The second case presented for voting consisted of a series of three low- and high-magnification images of a thymus from a female rat which contained a roundish, mostly encapsulated, expansile mass (Figure 7E, arrowheads) with a cytologic pattern differing from that of the adjacent unaffected thymus, including multiple small pale staining cellular clusters (Figure 7E, arrows). Voting choices made available to the audience were normal, benign thymoma, malignant thymoma, malignant lymphoma, epithelial hyperplasia, medullary hyperplasia, mononuclear cell leukemia, and other. The majority of the voting attendees chose either *benign thymoma* (36%) or *epithelial hyperplasia* (27%). The other diagnoses received from 0 to 18% of the votes: normal (12%), malignant thymoma (2%), malignant lymphoma (1%), medullary hyperplasia (18%), mononuclear cell leukemia (0%), and other (2%). Again, the speaker’s preferred diagnosis was benign thymoma.

After voting, low- and high-magnification images of cytokeratin-18 immunohistochemical images of case 2 were presented. These images served two purposes; first, these images show the increased density of cytokeratin-18 immunopositive cells within the tumor, and second, the images allow better visualization of the fibrous capsule surrounding the proliferative lesion, providing further evidence that this lesion in fact was a tumor (Figure 7F).

During the discussion of case 2, low- and high-magnification images from larger thymomas from female rats, with a more developed medullary differentiation pattern, were shown to the audience. Case 2 did exhibit medullary differentiation though not to the extent as some of the larger tumors; however, this case was chosen for its smaller size which provided more of a diagnostic dilemma to the PWG and hopefully to the symposium attendees. The medullary pattern still consists of abundant lymphocytes, though these cells are often found cuffing variably-sized clusters of pale-staining cells (Figure 7G, arrowheads; Figure 7H, higher magnification). This pattern has also been referred to as having an “organoid” appearance, and often contains numerous thin fibrous trabeculae subdividing the tumor into lobules (Kuper and Beems 1990).

Two examples of predominantly epithelial tumors from male rats from this same study were also shown to the audience. The one example of a malignant thymoma was shown at low- and high-magnification (Figures 7I and 7J, respectively), and this tumor was a nice example

of a thymoma with large ovoid epithelial cells exhibiting some extent of squamous differentiation. Residual thymus tissue is indicated by the arrows in panel I. A high-magnification image of a predominantly epithelial thymoma with both ovoid and spindle-cell populations was also shown to illustrate this potential pattern (Figure 7K).

An additional point made during the presentation was that thymomas can have multiple cytologic patterns, as depicted in Figure 7L. This particular thymus from a female rat has three distinct tumor appearances, potentially representing three discrete tumors, with a small amount of residual, atrophied thymus at the bottom of the panel (arrows).

A pre-neoplastic lesion that precedes thymoma development is not currently described in the literature. A lesion has been described in older rats in which the thymus is persistent or hyperplastic, the organ larger than normal (Kuper and Beems 1990). However, this process involves the entire organ, whereas thymomas are typically focal, and the normal cortical and medullary architecture is preserved, unlike in thymomas where there is distorted architecture (Kuper and Beems 1990). In one reference, the authors discuss that there is a considerable spectrum of thymic proliferative lesions, resulting in difficulty in applying a clear cut borderline diagnosis between hyperplasia and neoplasia (Kuper et al. 1986). The authors suggest, then, that lesions with at least one feature characteristic of a thymoma should be considered neoplastic (Kuper et al. 1986).

Charles River Wistar Han (CrI:WI[Han]) rats were used for a small number of studies within the NTP. Three of the studies had full peer review evaluation of the thymus for varying diagnoses. During the review of the thymus, a number of small to large proliferative lesions of varying cytologic appearance were noted. In the particular study from which the cases were selected for this presentation, fifteen representative thymuses were evaluated by a PWG for the presence of a thymoma (Mann and Hardisty 2014). This lesion engendered considerable discussion due to the variability of the tumor appearance, the similarity to malignant lymphoma in some instances, and the small size of the lesions in others.

Thymomas are relatively uncommon spontaneous lesions in aged Wistar Han rats (Giknis and Clifford 2011; Taylor et al. 2012). The March 2011 compilation of 2-year study historical control data from the supplier of the rats in this study indicates that benign and malignant thymomas have an incidence range of 1.54–4.17% and 0.89–5% in males, respectively; and 1.67–6.67% and 0.83–5% in females, respectively (Giknis and Clifford 2011). A total of 16 thymic tumors (10 benign, 6 malignant) were observed in 1217 control males, and a total of 48 thymic tumors (33 benign, 15 malignant) were observed in 1217 control females. Thymomas are tumors of the thymic epithelial cells; however, these tumors can have many different appearances as some can have a predominant lymphoid population and others can have a predominant epithelial population (Kuper and Beems 1990; Taylor et al. 2012). Thymomas are also relatively uncommon in other Wistar rat strains (Kuper et al. 1986; Poteracki and Walsh 1998; Walsh and Poteracki 1994), and are seen at very high incidence in Wistar/Neuherberg (W/Nhg) rats (Murray et al. 1985), and in Buffalo/Mna rats (Taguchi et al. 1992).

Thymomas in man have been associated with autoimmune disorders, particularly myasthenia gravis (MG). It is estimated that 10–15% of patients with MG have thymomas (Blalock et al. 1939; Good 1947; Keynes 1946; Souadjian et al. 1974). In one retrospective review, 10 of 35 patients with MG had thymomas, and of the remaining 25 patients, 23 were under the age of 34 (early onset MG), and 19 had germinal center formation in the thymic medulla (Castleman and Norris 1949). Similarly, there is a likely association of thymoma with motor dysfunction in BUF/Mna rats and a high prevalence of predominantly lymphocytic thymomas in this rat strain (Kato and Watanabe 1982; Taguchi et al. 1992). This strain also is affected with a nephritic syndrome; however, thymectomized rats still develop severe kidney disease and thus there appears to be no association with the high incidence of spontaneous thymomas (Nakamura et al. 1988).

ANGIOMATOUS LESIONS IN MESENTERIC LYMPH NODES

Dr. Schantel Hayes (Charles River Laboratories, PAI, Durham, NC) presented angiomatous lesions in the mesenteric lymph nodes in Wistar Han (CrI:WI[Han]) rats from an unpublished NTP chronic inhalation toxicity and carcinogenicity bioassay, the same studies that were presented by Dr. Jessica Hoane. The pathologist involved in the review of this study included the NTP pathologist (Dr. Gordon Flake, NTP, NIEHS, RTP, NC), the study pathologist (Dr. Lauren Staska, Battelle Memorial Institute, Columbus, OH [currently of WIL Research]), the quality assurance (QA) pathologist (Dr. E. Terrence Adams, formerly of EPL, RTP, NC) and the pathology working group (PWG) coordinator (Dr. Jessica Hoane, Charles River Laboratories, PAI, Durham, NC).

Male and female Wistar Han rats were dosed via inhalation for 105 weeks in a two year bioassay, and had an increased incidence of vascular lesions in the mesenteric lymph nodes. The first case presented for voting consisted of a series of low- and high-magnification images of a vascular lesion; this lesion completely effaced the lymph node architecture and was composed of proliferating blood vessels supported by a fibrovascular stroma. Vessels were variably sized, usually thin walled, lined by plump endothelial cells and contained red blood cells. Solid areas contained collapsed or poorly formed irregularly shaped, anastomosing vascular spaces (Figure 8A). Neoplastic cells lining vascular channels or spaces were large and pleomorphic with round to elongate nuclei containing a hyperchromatic chromatin pattern and indistinct nucleoli. Mitotic figures were frequent. Voting choices and results were hemangioma (12%), hemangiosarcoma (63%), vascular transformation (4%), endothelium hyperplasia (1%), angiomatous hyperplasia (14%), vascular malformation or hamartomas (6%), and other (1%). The majority vote was for *hemangiosarcoma*, which was also the speaker's choice.

A second vascular lesion from the same study was presented as case two in a series of low- and high-magnification images. The mesenteric lymph node contained an expansile mass that effaced the majority of the architecture and compressed a small band of remnant lymphocytes at its proximal border. Microscopically, the proliferation was composed of variably sized cavernous spaces lined by a single layer of well differentiated neoplastic cells (Figure 8B). Neoplastic cells contained elongate nuclei with a hyperchromatic chromatin pattern and indistinct nucleoli. Voting choices and results were hemangioma (69%),

hemangiosarcoma (8%), vascular transformation (1%), endothelium hyperplasia (1%), angiomatous hyperplasia (10%), vascular malformation or hamartomas (11%), and other (1%). The majority vote was for *hemangioma*, which was also the speaker's choice.

Two additional angiomatous lesions were presented as cases three and four and were considered more challenging diagnostic cases. Both cases had a series of low- and high-magnification images and represented a similar process that differed only in the extent of lymph node involvement. Case three was a focal, well-demarcated lesion (Figure 8C) while case four was focally extensive involving a larger portion of lymph node architecture (Figure 8D). Both proliferative lesions contained an increased number of endothelial cells and blood filled vessels and spaces supported by varying quantities of fibrovascular stroma (Case 3, Figure 8E; Case 4, Figure 8F). In both cases endothelial cells lacked cellular pleomorphism and lacked nuclear atypia. Mitotic figures were not present. Voting choices and results for case 3 were hemangioma (9%), hemangiosarcoma (29%), vascular transformation (3%), endothelium hyperplasia (11%), angiomatous hyperplasia (37%), vascular malformation or hamartomas (8%), and other (3%). *Angiomatous Hyperplasia* received the majority of votes and this was also the speaker's diagnostic choice. Voting choices and results for case four were hemangioma (5%), hemangiosarcoma (41%), vascular transformation (4%), endothelium hyperplasia (13%), angiomatous hyperplasia (21%), vascular malformation or hamartomas (15%), and other (2%). As for case 3, angiomatous hyperplasia was the speaker's diagnostic choice. However, *hemangiosarcoma* garnered the most votes despite the lesion's histomorphologic similarities to case three. This choice in diagnosis may have been due to the more extensive involvement of tissue (Figure 8D) rather than the cellular morphology. There is an apparent invasion into the surrounding adipose tissue, however this is most likely the normal infiltration of adipose tissue as seen in age-related physiological involution, normally seen in a two-year bioassay.

Dr. Hayes reviewed the morphologic diagnostic criteria used to categorize these angiomatous lesions (Table 2). The nomenclature and diagnostic criteria described were based on previous publications and have been widely used to diagnose angiomatous proliferations (Hardisty et al., 2007). Angiomatous hyperplasia is a non-neoplastic, well-demarcated lesion consisting of an increased number of capillaries, other vascular structures and varying quantities of fibrovascular stroma. Uniform vascular spaces are generally blood filled. Endothelial cells are normal-appearing and there are no mitotic figures. Hemangiomas are focal, non-invasive, circumscribed nodular masses composed of variably sized cavernous spaces lined by a single layer of well-differentiated endothelial cells. Hemangiosarcomas are composed of an increased number of endothelial cells that form an anastomosing meshwork of blood filled channels of varying size. Endothelial cells can be multi-layered and are often pleomorphic, enlarged, and contain hyperchromatic nuclei. Solid areas with collapsed vascular channels and areas of necrosis are common.

The slides of all mesenteric lymph node angiomatous lesions were examined and diagnosed by six NTP pathologists, and images from a select number of lymph nodes were reviewed and diagnosed by three consultants with experience in rodent vascular lesions. The participating pathologists were requested to categorize these lesions as benign or malignant based on morphologic criteria. There was lack of a clear consensus in all but one lymph

node lesion, illustrating the difficulty of categorizing these lesions based on morphologic criteria alone (Table 3).

Mesenteric lymph node angiomatous lesions have not previously been identified in NTP studies, possibly due to the use of the F344/N rat default strain. There was a temporary change to the Wistar Han rat, before the final default strain (HSD: Sprague Dawley SD) was used (King Herbert et al. 2010). These lesions are rarely observed as a spontaneous lesion in F344 and F344/N rats (Haseman et al., 1998, Tennekens et al., 2004, Radi and Morton, 2012). However, mesenteric lymph node angiomatous lesions are reportedly a common spontaneous lesion in aged Wistar Han rats (Deerberg et al., 1980, Reindel et al., 1992, Tucker, 1997, Weber et al., 2011). They occur at a higher incidence in males and occur more frequently in outbred strains such as the Sprague Dawley and other Wistar strains (higher incidence in female BASF Wistar females) when compared to inbred F344 rats (Deerberg et al., 1980, Haseman et al., 1998, Radi and Morton, 2012, Reindel et al., 1992, Tennekens et al., 2004, Tucker, 1997, Weber et al., 2011, Creasy, 2012). Controversy has existed over how to morphologically classify these angiomatous proliferations as benign or malignant and the biologic behavior of these lesions is unknown (Reindel et al., 1992, Tucker, 1997). These lesions are generally incidental at necropsy and distant metastasis has not been reported (Tucker, 1997, Creasy, 2012, Reindel et al., 1992).

During the discussion period, one audience member asked about the progression of these lesions from a non-neoplastic change, such as angiomatous hyperplasia, to hemangioma or hemangiosarcoma. At the time of presentation, a comprehensive study on the biologic behavior of these lesions had not been performed, therefore Dr. Hayes did not have a scientifically sound answer. Another audience member noted that a continuum of these lesions from a benign to a malignant proliferation (hyperplasia to hemangiosarcoma) has been observed within his laboratory. Another audience member noted that diagnostic difficulty often lies in differentiating angiomatous hyperplasia from hemangiosarcoma. High grades of angiomatous hyperplasia can be difficult to differentiate from tumors. Both lesions can be morphologically similar in terms of areas of proliferating endothelial cells and blood filled vessels/spaces (Creasy, 2012). Lastly, an audience member raised concern about diagnosing these proliferations as malignant without evidence of metastasis. Dr. Hayes agreed with the audience member and stated that only local invasion outside of the lymph node has been found, but no metastasis (Tucker, 1997, Reindel et al., 1992). Furthermore, Dr. Hayes stated that the criteria used to diagnose these lesions as malignant in this study primarily includes nuclear atypia and the marked variation of vascular spaces from small rounded to anastomosing, collapsed and irregular.

AN UNUSUAL RETINAL LESION OF THE FOVEA

Dr. Susan A. Elmore (NTP, NIEHS, Research Triangle Park, NC, USA) presented an unusual retinal foveal lesion on behalf of Dr. Haruko Koizumi and her collaborators Drs. Akihito Shimoi and Hirofumi Hatakeyama (Ina Research Inc., Nagano, Japan).

Dr. Elmore began her presentation with an overview of the structure and function of the fovea centralis (Kolb 2014; Schafer and Render 2012). This structure is present in many

types of fish, reptiles, and birds, but is not present in the rodent retina. There are many species differences in ocular structures, but there is quite a bit of similarity between monkey and human eyes, including the presence of a fovea. Among mammals, it is found only in primates. Simian primates, marmosets, and squirrel monkeys are used as models of foveal disease (Hendrickson 1992).

Fovea centralis, generally known as the fovea, is a Latin term that means “pit”, which alludes to the visible depression in the retina at this region (Figure 9A). It is located in the center of the macula lutea region of the retina, is about 1.5mm in diameter, and contains the largest concentration of cone cells in the eye, thus it is responsible for sharp central vision (Provis 2001). This region does not have rods and is not sensitive to dim lights. If an object is large, one must constantly shift ones gaze to bring different portions of an image into the fovea. Half of the nerve fibers in the optic nerve carry information from the fovea while the remaining half carry information from the rest of the retina. The fovea comprises less than 1% of the retinal size but takes up over 50% of the visual cortex in the brain. Progressive destruction of this region is called macular degeneration, which results in a loss of central vision.

The fovea centralis is about 1.5mm wide and it is composed of four distinct regions: foveola, fovea, parafovea, and perifovea (Iwasaki and Inomata 1986). The central foveola is about 2–35mm in diameter, is avascular, and contains only cone photoreceptors and Müller cells. Surrounding the foveola is the fovea, which is also avascular. The avascular nature of these two inner foveal regions allows light to be sensed without any dispersion or loss. Surrounding the fovea is the intermediate parafovea belt, which contains a ganglion cell layer with more than 5 rows of cells. And surrounding the parafovea is the outer perifovea region, which contains fewer ganglion cells, about 2–4 rows. The foveal pit is surrounded by the foveal rim, which contains the displaced neurons from the pit and is therefore the thickest part of the retina.

The case for voting was from a control 2-year-old cynomolgus monkey. This was a one-month toxicity study with a one-month recovery period. The animal was dosed with methylcellulose vehicle. The audience was shown representative images (Figures 9B–D) and asked to vote. The voting choices and answers were artifact (21%), edema, fovea (17%), atrophy, fovea (1%), cystic degeneration, fovea (36%), vacuolar degeneration, fovea (24%), and other (1%). The audience members’ first choice was *cystic degeneration* and this was also the favored diagnosis of the contributors. Notably, 79% felt that it was a lesion and 21% felt that it could be artifact.

This monkey was from a control group in a 4-week toxicity study. This lesion was not detected ophthalmologically, but was found histologically. It was bilateral in this particular animal but was not present in other monkeys in this study. The control animals from this study had no changes in the optic nerve and a normal retina with a normal macula lutea, including the fovea centralis. In the affected animal the optic nerve was also unaffected and the lesion was confined to the fovea centralis (Figure 9B). Within the fovea centralis, the photoreceptor cell layer and inner and outer plexiform layers were vacuolated and

hypertrophied with larger cystic spaces within the inner plexiform layer of the fovea (Figures 9C and 9D). The vacuolization may be consistent with edema.

There was discussion about whether or not this lesion could be an artifact. The fixative was 2.5% glutaraldehyde and 1% formaldehyde in phosphate buffer. Differential rates of fixation may lead to vitreous shrinkage and tugging on the retina, and this would most often be seen at the fovea.

Idiopathic optic neuropathy was also considered as a potential etiology. This lesion is seen in rhesus and cynomolgus macaques and consists of a spectrum of lesions: reduction of ganglion cells in the macular region; thinning of the temporal retinal nerve fiber layer due to axonal loss; temporal pallor of the optic disc; loss of axons and gliosis of temporal optic nerve; and spaces within the inner nuclear layer of the temporal retina.

Cystoid macular edema was another differential for this lesion. This is a vision impairing but reversible phenomenon in medical ophthalmology. The cause is usually ocular inflammation. A comparison of images this lesion to those of confirmed cystoid macular edema found that none had exactly this type of cavitated change but they all had a breakup of the tissues at or near this level (some were more in the inner and outer segments). Bilateral optic atrophy is another differential, but arguing against this diagnosis is the lack of ganglion cell pathology in the presented lesion.

Another comment (post meeting) was that this lesion is reminiscent of a “macular hole”, which is a small break in the macula (Gass 1999). In aging human patients, the vitreous generally thickens and can shrink and pull away from the macula. If the gel sticks to the macula without pulling away, then the macular tissue stretches and eventually tears, forming holes. The fluid that has replaced the shrunken vitreous can then seep through the hole and into the tissues of the macula. The cuffing around the foveal retinal depression may be due to vitreous traction of the retina. There is a 10–15% chance that macular holes can be bilateral (<http://www.nei.nih.gov/health/macularhole/macularhole.asp>, retrieved 7/14/14).

There is another condition called “vitreomacular traction syndrome”, characterized by an incomplete posterior vitreous detachment with the persistently adherant vitreous exerting tractional pull on the macula (http://eyewiki.aao.org/Vitreomacular_Traction_Syndrome#Clinical_diagnosis, retrieved 7/14/14). This condition is usually diagnosed by optical coherence tomography, which shows foveal cystoid changes involving the inner retina and preservation of the outer retinal morphology.

A similar lesion of fovea cystic degeneration has been found in 4 monkeys in other studies, all were 4–5 years old, and all have been from the same breeder in the Philippines. The lesion was present in the treated groups, but it was not judged to be treatment-related. Rather it was considered to be a spontaneous background change. Comments from the audience were to mostly agree that it is not likely artifact and that this company should pay special attention to this region of the eyes from future studies and record whenever present since there may be an underlying genetic factor involved.

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NOMENCLATURE CHALLENGES FROM THE ENDOCRINE INHAND OWG

The Endocrine INHAND OWG identified three examples of nomenclature challenges for endocrine lesions in rodents that were presented for voting and discussion. Dr. T. Rosol (OWG chair) made the presentation and the cases were selected and organized with the help of other OWG members (Drs. A. Braendli-Baiocco (co-chair), E. Balme, M. Bruder, S. Chandra, J. Hellmann, M. Hoenerhoff, B. Lenz, M. Mense, T. Kambara, S. Rittinghausen, H. Satoh, F. Schorsch, F. Seelinger, M. Tsuchitani, and Z. Wojcinski).

The first case involved the nomenclature of focal adrenal cortical lesions. The OWG has found it particularly difficult to develop consensus for diagnostic criteria for the following five diagnoses; (1) vacuolation, decreased, focal; (2) vacuolation, increased, focal; (3) hypertrophy, focal; (4) hyperplasia, focal; and (5) adenoma. Diagnostic criteria often overlap between the five diagnoses (Laast *et al.* 2014; Rosol *et al.* 2013). There is usually a lack of consistency and varying nomenclature used between pathologists for these types of lesions. The goal of the OWG is to define diagnostic criteria that provide a guideline to enable pathologists to more uniformly use preferred diagnostic terms.

Initially, images of three focal adrenocortical lesions were presented for interpretation and voting. These three cases involve a spontaneous change/lesion that occurs in the adrenal cortex of young or aged rats of multiple strains. The diagnostic options, voting results, and preferred terminology by the OWG were as follows (Figures 10A, 10B, and 10C):

- Case 10A: Hyperplasia, focal (30%); Hypertrophy, focal (7%); Cellular alteration, focal (12%); Cellular atypia, focal (3%); Basophilic focus of alteration (28%); Eosinophilic focus of alteration (9%); Degeneration, focal (0%); Decreased vacuolation, focal (10%; **preferred OWG diagnosis**). After discussion a majority of participants considered that *focal hyperplasia* or *focal decreased vacuolation* were the most appropriate diagnoses. The INHAND Endocrine OWG will develop criteria to facilitate differentiation of focal cortical hyperplasia and focal decreased vacuolation; however, it is anticipated that there will continue to be overlap between these two diagnoses.
- Case 10B: Hyperplasia, focal (16%, **preferred OWG diagnosis**); Hypertrophy, focal (42%); Cellular alteration, focal (6%); Cellular atypia, focal (8%); Basophilic focus of alteration (1%); Eosinophilic focus of alteration (13%); Degeneration, focal (9%); Decreased vacuolation, focal (4%). After discussion, *focal hyperplasia* or *focal hypertrophy* were the most accepted diagnoses.

- Case 10C: Hyperplasia, focal (4%); Hypertrophy, focal (13%); Cellular alteration, focal (7%); Cellular atypia, focal (1%); Clear cell focus (15%); Eosinophilic focus of alteration (2%); Degeneration, focal (1%); *Vacuolation, increased, focal* (57%; **preferred OWG diagnosis**); Other (1%).

As can be seen by the voting results, there was wide variability in interpretation of the lesions by the membership and initial consensus on only one of the three cases. This emphasized the need for standardization of diagnostic criteria. Lively discussion with some general agreement and continued differing opinions followed.

Initially, the tentative nomenclature proposal of the OWG was presented. The OWG suggested that the diagnosis, **Hyperplasia, cortical, focal**, be used for lesions where the pathologist interprets that the nonneoplastic focal lesion has an increase in cortical cell number with no or minimal compression of adjacent cortical cells. This included foci of hyperplasia with large or small cells and eosinophilic or basophilic cells. It was noted that interpretation of increased cell number is challenging when the cells are larger than normal, because this may be associated with reduced nuclear density. In addition, focal hyperplasia is usually rounded in outline, vacuolation may be present, cells and nuclei may have atypia, the cortical architecture is usually maintained, and the foci are not larger than the cortical width. It was proposed that the diagnoses of cellular alteration, clear cell focus, eosinophilic focus, and basophilic focus not be used for **hyperplasia, cortical, focal** in the future. There was general consensus on this, which will significantly reduce the number of diagnoses for focal adrenocortical lesions.

Markers for proliferation (Ki-67, PCNA, and BrdU) can be useful to confirm a diagnosis of hyperplasia, even though they are not used routinely. The Registry of Industrial Toxicology Animal-data (RITA) contains a large series of BrdU-labeled rat adrenals from a 2-year carcinogenicity study (courtesy of BASF SE and Dr. B. Kittel). The BrdU-labeling of the adrenals was incidental to the study design, but proved useful to identify proliferating adrenocortical cells in foci of hyperplasia. Most foci that were identified as focal hyperplasia or hypertrophy contained BrdU-labeled nuclei in the outer regions of the nodule and few labeled nuclei towards the medulla (Figures 10D and 10E). This suggested to the OWG that the diagnostic term “focal hypertrophy” may actually represent focal hyperplasia, large cell type. After discussion at the NTP Satellite Symposium, it was generally concluded that diagnoses should be based on the morphology of cortical cells in an H&E-stained slide and focal hypertrophy should remain an available diagnosis. Future studies of focal adrenocortical nodules in rats using proliferation markers is encouraged in order to gain additional understanding on the incidence of focal hyperplasia (large cell type) and focal hypertrophy.

Case 2 was a spontaneous thyroid lesion that occurs in male or female Wistar Hannover GALAS rats (Figure 10F). The lesion may be seen in normal or stunted rats, but usually occurs as an incidental finding in short- or long-term toxicity studies. The cellular features are enlarged follicular cells (hypertrophy) with a large basilar cytoplasmic vacuole containing eosinophilic proteinaceous fluid and resulting in apical displacement of the nucleus. Ultrastructurally, the vacuole consists of dilated endoplasmic reticulum (Sakai *et al.*

2000). The voting results for the diagnoses were: Thyroid dysplasia (10%, **current preferred OWG diagnosis**); Intracytoplasmic edema, follicular cell (5%); Vacuolation, follicular cell (14%); Vacuolar degeneration, follicular cell (5%); Vacuolation with nuclear displacement (16%); Dilated endoplasmic reticulum, follicular cell (3%); Hypertrophy, follicular cell (32%); Hypertrophy and vacuolation, follicular cell (15%).

The OWG currently prefers the diagnosis of thyroid dysplasia because this is the terminology used in the literature that describes the disease in rats (Sakai *et al.* 2000; Shimoi *et al.* 2001; Takaoka *et al.* 1994; Weber *et al.* 2009). The disease is due to an unidentified but heritable genetic abnormality in Wistar Hannover GALAS rats. Homozygous rats have thyroid dysplasia, stunted growth, reduced pituitary gland growth hormone (GH) and are hypothyroid. Heterozygous rats have thyroid dysplasia but are euthyroid (Weber *et al.*, 2009). It has been reported that there was no increase in follicular cell hyperplasia or neoplasia, but the homozygous rats may be more sensitive to carcinogens (Abe *et al.* 2012). Dwarf or stunted rats are hypothyroid with increased circulating thyroid stimulating hormone (TSH) (Doi *et al.* 2004). Increased focal follicular cell hyperplasia and neoplasia was reported in dwarf rats over 50 weeks of age (Kokoshima *et al.* 2014). This disease was thought to be limited to GALAS rats; however, it has also been reported to occur spontaneously in cynomolgus monkeys (Hatakeyama *et al.* 2011).

Case 2 was presented for discussion because the current diagnosis, thyroid dysplasia, does not describe the morphology of the lesion. It was debated whether the diagnosis should be modified to one that is more descriptive of the pathology. After the discussion, the membership was divided in their opinion. The second vote resulted in the following distribution of diagnoses: Thyroid dysplasia (27%); Intracytoplasmic edema, follicular cell (0%); Vacuolation, follicular cell (26%); Vacuolar degeneration, follicular cell (6%); Vacuolation with nuclear displacement (23%); Dilated endoplasmic reticulum, follicular cell (0%); Hypertrophy, follicular cell (5%); Hypertrophy and vacuolation, follicular cell (4%). Most (two-thirds) favored a descriptive diagnosis (*vacuolation or vacuolation with nuclear displacement*); however, one-third of the voters still favored the diagnosis of the entity (*thyroid dysplasia*), mainly because it is a very specific genetic lesion of the Hannover GALAS rat.

Case 3 was presented as an unusual spontaneous lesion that occurs in young or old rats in the pars nervosa of the pituitary gland (Figures 10G and 10H). The lesion is characterized by acinar, tubular, or fusiform glandular structures in the neurohypophysis or between the pars intermedia and pars nervosa (Iwata *et al.* 2000; Schaetti *et al.* 1995). The structures are often associated with cysts of Rathke's cleft. The epithelial cells have eosinophilic cytoplasm and basal nuclei. The cytoplasm contains eosinophilic secretory granules that are PAS-positive and Alcian blue-negative. The glandular structures are morphologically similar to parotid salivary glands and ultrastructurally myoepithelial cells are seen surrounding the epithelial cells (Iwata *et al.* 2000).

The voting results for the diagnoses were: Craniopharyngeal dysplasia (8%); Craniopharyngeal derivatives (9%); Aberrant craniopharyngeal structures (27%, **preferred OWG diagnosis**); Craniopharyngeal cysts (6%); Craniopharyngeal duct hyperplasia (33%);

Craniopharyngeal duct metaplasia (8%); Craniopharyngioma (7%); Other (2%). In general, there was little controversy over the diagnosis. Discussion centered on whether the lesion was a developmental anomaly or an acquired hyperplasia/metaplasia. It was agreed the lesion does not represent a neoplasm even though there is apparent invasive behavior into the pars nervosa. It was concluded that the preferred diagnosis is *aberrant craniopharyngeal structures*, based on the current literature.

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Abbreviations

AB	Alcian blue
ATLS	acute tumor lysis syndrome
CCSP	club (Clara) cell-specific protein
COPD	chronic obstructive pulmonary disease
DIVI	drug induced vascular injury
CrI	WI[Han], Wistar Han
GESC	global editorial steering committee
GH	growth hormone
GLP	good laboratory practice
H&E	hematoxylin & eosin
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria
MG	myasthenia gravis
NO	nitric oxide
NTP	National Toxicology Program
OWG	Organ Working Group
PAS	periodic acid Schiff
PEMD	proestrus, estrus, metestrus, diestrus
PDE	phosphodiesterase

PWG	pathology working group
QA	quality assurance
RITA	Registry of Industrial Toxicology Animal-data
SPC	surfactant protein C
STP	Society of Toxicologic Pathology

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Figure 1.

Mucinous adenoma in the lung of an untreated control male B6C3F1 mouse. Low power view of lung mass (A, arrow). Note adjacent bronchus (Br). Bv = Blood vessel (H&E). Higher magnification of lung mass (B). Lung mass consists primarily of acinar-like structures (a) lined by non-ciliated, columnar epithelial cells with abundant, pale bluish-pink cytoplasm and generally small, uniform, basal nuclei arranged in single rows. Supporting fibrous stroma (f) exhibits a mixed inflammatory cell infiltrate (H&E). Figure C shows acinar cells that exhibit strongly positive cytoplasmic staining with periodic acid Schiff (PAS) reaction with diastase (left) (magenta) and Alcian blue at pH 2.5 (right) (turquoise). Most cells lining acinar structures (a) in Figure D are negative for CCSP (club [Clara] cell-specific protein). Only a few scattered single cells exhibit CCSP-positivity (short arrow). Note rare mitotic figure (long arrow). Cells lining acinar structures (a) in Figure E are negative for SPC (surfactant protein C). Mucinous cells in Figure F extend along existing

alveolar septa (lepidic growth) (arrows) at border of mass (M) (H&E). Alveolar parenchyma (AP). b = bronchioles entrapped at periphery of mass.

Figure 2.
Plexiform vasculopathy in Wistar Han rats.

Low (A) and high (B) magnification photomicrographs from a large mediastinal vessel. Note the formation of numerous variably-sized and shaped endothelial-lined vascular spaces expanding the tunica media of the vessel. Figures C and D are low and high magnification photomicrographs (respectively), of a mesenteric vessel, likely a vein, with numerous tortuous, incomplete endothelial-lined vascular spaces expanding/dissecting through the tunica media. Little inflammation was associated with these vascular lesions, and the endothelial cells lining the spaces in panels are relatively flattened and quiescent with no evidence of pleomorphism or mitotic figures. H&E

Vascular changes seen more commonly within the current study are illustrated in Figures E–G from the kidney and Figure H from the mediastinum. Lesions include medial changes such as vacuolation (E, arrows), cytoplasmic necrosis and hemorrhage (E and H, white arrowheads), subintimal deposition of serum proteins which is often referred to as “fibrinoid necrosis” (F and G, arrows) and varying severity of adventitial inflammation (Figures E–H, black arrowheads). H&E

Figure 3.

Rodent vaginal cytology

(A) Many neutrophils (arrows), characterized by their very small size and multilobulated nuclei, and two large nucleated epithelial cells (arrowhead) are present on this diestrous vaginal smear from a Sprague Dawley rat (Toluidine blue stain). (B) Small nucleated epithelial cells from a proestrous vaginal smear of a Sprague Dawley rat. Lower numbers of anucleated epithelial cells are also seen (right center) (Toluidine blue stain). (C) Several large nucleated epithelial cells (arrows) are intermixed with anucleated epithelial cells (arrowheads) from a Sprague Dawley rat vaginal smear of late estrus. Bacteria are adhered to many of the epithelial cells (Modified Wright–Giemsa stain). (D) Anucleated epithelial cells from a vaginal smear of estrus in a B6C3F1/N mouse. Some cells possess ghost nuclei (arrows) (Modified Wright – Giemsa stain). (E) Vaginal smear of proestrus in a Sprague Dawley rat characterized by high numbers of small nucleated epithelial cells found

individually and in cohesive clusters (arrow) (Toluidine blue stain). (F) Vaginal smear of estrus in a Sprague Dawley rat characterized by high numbers of anucleated epithelial cells (Modified Wright – Giemsa stain).

(G) Vaginal smear of late estrus in a Sprague Dawley rat characterized by the presence of round, oval and spindle shaped nucleated epithelial cells interspersed among anucleated epithelial cells (Modified Wright – Giemsa stain). (H) Vaginal smear of metestrus in a B6C3F1/N mouse. Neutrophils are seen among anucleated epithelial cells (Modified Wright – Giemsa stain). (I) Vaginal smear of metestrus in a Sprague Dawley rat. Neutrophils, small and large nucleated epithelial cells and anucleated cells are all present (Toluidine blue stain). (J) Vaginal smear of metestrus in a B6C3F1/N mouse. At the peak of metestrus, a flood of neutrophils is seen among the anucleated epithelial cells resulting in smears of high cellularity (Toluidine blue stain). (K) Vaginal smear of diestrus in a Sprague Dawley rat. Neutrophils are widely scattered among low numbers of epithelial cells (Modified Wright–Giemsa stain). (L) A low cellularity diestrous vaginal smear in a B6C3F1/N mouse consisting of scattered neutrophils and epithelial cells. Modified Wright – Giemsa stain.

Figure 4.

Pancreatic lesions from a male Sprague Dawley rat. (A and B) Pancreas sections at low and higher magnifications from a male Sprague Dawley rat treated with test-article. Note the locally extensive, perilobular to lobular inflammation and fibroplasia. (C) Atrophic pancreatic acini from the same section with mononuclear to mixed inflammation and fibroplasia. (D) Affected pancreatic islet from the same section with hemorrhage, hemosiderin containing macrophages, inflammation and fibroplasia. H&E

Figure 5.

Retinal dysplasia in Wistar Han rats and B6C3F1 mice.

(A) Wistar Han rat eye with disorganized, circular folding of retinal layers consistent with retinal dysplasia. (B) B6C3F1 mouse eye with similar histologic features as that seen in Figure A. (C) Coronal section of developing eye from a mouse embryo day 17.5. Note immature appearance of the retinal layers. H&E

Figure 6.

(A–E) Acute tumor lysis syndrome (ATLS) in a male Tg.ras H2 mouse with disseminated lymphoma. There are multifocal intravascular clumps (emboli) of basophilic material (arrow) in lungs, adrenal glands, kidneys, liver and heart. These emboli are either admixed with fibrillary eosinophilic material in lungs (A) or observed as elongated homogenous, acellular and basophilic microemboli in small vessels of glomeruli, adrenal cortex, liver and heart (B–E). (F–H) Skeletal muscle (thigh) of female Tg.rasH2 mouse with skeletal myopathy. At low magnification (F), there are multifocal to coalescing areas of muscle degeneration admixed with inflammatory infiltrate. Inflammatory cells include neutrophils, lymphocytes, and macrophages (G). The regenerating fibers showed basophilic cytoplasm and nuclear rowing (H) (H&E).

Figure 7.

Benign thymomas in Wistar Han rats.

Case 1, an affected thymus from a female rat was subgrossly characterized by a roundish, unencapsulated mass with smooth margins that had totally effaced the normal thymus architecture (A). At higher magnification (B) the mass was predominantly composed of small lymphocytes with dark basophilic, densely condensed nuclei and little cytoplasm that somewhat masked an underlying population of larger epithelial cells (B, arrows), with large vesicular nuclei and moderate to abundant pale eosinophilic cytoplasm. Cytokeratin-18 immunohistochemically stained slides of a control thymus (C), and the same thymus depicted in Figures A and B (D), illustrates the increased epithelial cell population, which has brown cytoplasmic staining within the tumor as compared to the control. Case 2, an affected thymus from a female rat contains a roundish, mostly encapsulated, expansile mass (E, arrowheads) with a cytologic pattern differing from that of the adjacent unaffected thymus, including multiple small pale staining cellular clusters (E, arrows). Cytokeratin-18

immunohistochemically stained slides (F) of the tumor from 7E reveal increased numbers of cytokeratin-18 immunopositive cells within the tumor (demarcated by arrowheads), and better highlights the fibrous capsule surrounding the tumor.

Low- (G) and high- (H) magnification images are from larger thymomas from female rats with a medullary differentiation pattern, which consists of abundant lymphocytes, often found cuffing variably-sized clusters of pale-staining cells (G, arrowheads; H, higher magnification). Low magnification image (I) from a malignant thymoma from a male rat, depicting a predominant ovoid neoplastic epithelial cell population effacing the thymus (arrows). Some extent of squamous differentiation is visible at higher magnification in this malignant thymoma (J). A benign, predominantly epithelial, thymoma from a male rat with both ovoid and spindle shaped neoplastic cells (K). Occasionally thymomas can have multiple cytologic patterns (L), though it is possible that this lesion from a female rat may represent three distinct tumors expanding separate lobules of the thymus, with a small amount of residual, atrophied thymus at the bottom of the figure (arrows).

Figure 8.

Angiomatous lesions in the mesenteric lymph nodes of male Wistar Han rats from a 2-year toxicity/carcinogenicity bioassay.

Figure A is a high magnification of a hemangiosarcoma from case 1. Neoplastic cells are arranged in variably sized vascular spaces that are small, round to anastomosing, and supported by a fibrovascular stroma. Figure B is a high magnification of a hemangioma from Case 2. This lesion is characterized by a proliferation of variably sized cavernous spaces lined by a single layer of well-differentiated neoplastic cells. Cases 3 and 4 are examples of angiomatous hyperplasia. Figure C shows the lesion from case 3 as a well-demarcated focal lesion, whereas Figure D shows the lesion from case 4 as a larger, more infiltrative lesion. Figures E and F show the similar tissue architecture and cellular morphology of cases 3 and 4, respectively. Both are characterized by a focally extensive non-neoplastic change composed of increased number of proliferating endothelial cells and blood filled vessels and

spaces supported by varying quantities of fibrovascular stroma. The endothelial cells lack cellular atypia.

Figure 9.

Retinal foveal lesion in a cynomolgus monkey.

Figure A is a normal fovea centralis (fovea) from a control animal. There is a slight depression in this area of the retina. Although not apparent by light microscopy, the central foveola region is avascular, contains only cone photoreceptors and Müller cells. Figure B is a low magnification image from one ocular globe of the affected animal. The optic nerve is within normal limits but there is hypertrophy and vacuolization of the various layers of the fovea. The sclera, choroid, and pigmented epithelial layer are all within normal limits.

Figures C and D are higher magnifications, showing the hypertrophy and vacuolization in the inner and outer plexiform and photoreceptor layers of the fovea centralis.

Figure 10.

Figures A–C illustrate focal lesions in the rat adrenal cortex. (A) Focal lesion with reduced cytoplasmic vacuolation and equivocal increased number of cortical cells. Consensus was not reached for this lesion. Preferred diagnoses were vacuolation, decreased, focal or hyperplasia, focal. (B) Hyperplasia, focal, large cell type with nuclear atypia. Many pathologists preferred hypertrophy, focal as the best diagnosis for this lesion. Adrenal capsule is on the left. (C) Vacuolation, increased, focal. There was a high degree of concurrence on this lesion. Figures D (H&E stain) and E (BrdU stain) illustrate hyperplasia, focal, large cell type, adrenal cortex, rat. Note the increased cell proliferation towards the outer or subcapsular region of the nodule with reduced cell proliferation towards the inner region of the nodule. Figure F illustrates thyroid dysplasia in a Wistar Hannover GALAS rat. Note the enlarged follicular cells (hypertrophy) with large basilar cytoplasmic vacuoles containing eosinophilic proteinaceous fluid and apical displacement of the nucleus. Images A–C are courtesy of Dr. M. Hoenerhoff and the NTP Archives. Images D and E are courtesy of BASF SE.

Figures 10G and 10H illustrate aberrant craniopharyngeal structures in a rat pituitary gland. (G) Aberrant glandular structures are present between the pars intermedia and pars nervosa (arrow) with infiltration into the pars nervosa. Note the Rathke's cleft cyst (arrowhead). (H). Aberrant glandular structures in the pars nervosa with similarity to the parotid salivary gland. A Rathke's cleft cyst is present on the left.

Table 1

Key Comparison Features of Select Pancreatic Injuries

Model/finding	Characteristics			
	Signalment	Inflammatory cell infiltrate	Acinar degeneration, necrosis, or atrophy	Islet hemorrhage/fibrosis
Exocrine injury	TA induced	Yes	Yes, diffuse	No
Endocrine injury	TA induced/ strain assoc.	Yes	Yes, peri-islet	Yes
Interface injury	TA induced, males and females	Yes	Yes, peri-islet and lobular	Yes
Background lobular atrophy	Old and young rats	Yes	Yes, lobular	No
Background islet hemorrhage and fibrosis	Older, mainly male SD rats	Yes	Yes, peri-islet	Yes
Current finding	Induced, 16 wk male SD rats	Yes	Yes, peri-islet and lobular	Yes

Table 2**Diagnostic Criteria for Mesenteric Lymph Node Angiomatous Lesions**

Lesion	Diagnostic Criteria
Angiomatous Hyperplasia	<ul style="list-style-type: none"> • Focal, well-demarcated, prominent lesion with increased number of capillaries and other vascular structures • Vascular spaces are usually blood filled • Absence of mitotic figures and atypia • Varying quantities of fibrovascular stroma
Hemangioma	<ul style="list-style-type: none"> • Focal, non-invasive expansion of capillary or sinusoidal structures • Variably sized cavernous spaces lined by a single layer of well-differentiated endothelium • Endothelial nuclei increased in number and may be slightly enlarged • Mitotic figures are infrequent
Hemangiosarcoma	<ul style="list-style-type: none"> • Discrete to irregular expansive mass with local invasion • An increased number of plump, pleomorphic endothelial cells with large hyperchromatic nuclei • Solid areas with collapsed vascular channels and areas with poorly formed, irregular-shaped vascular channels • Endothelial cells may be multilayered or form clusters • Mitotic figures are usually present

Table 3

Study Data

	Reviewing Pathologists									
	1	2	3	4	5	6	7	8	9	Total
LN1 ^a	M ^b	B ^c	M	B	B	B	B	B	B	7B, 2M
LN2	M	B	M	B	B	B	B	B	B	7B, 2M
LN3	M	B	B	B	B	B				5B, 1M
LN4	M	B	M	B	B	B				4B, 2M
LN5	M	M	M	B	B	M				2B, 4M
LN6	M	M	M	B	B	M	M	M	M	2B, 7M
LN7	M	B	B	B	B	M				4B, 2M
LN8	M	M	M	B	B	M				2B, 4M
LN9	M	B	M	B	B	M	B	M	M	4B, 5M
LN10	M	B	M	B	M	M				2B, 4M
LN11	M	B	M	B	M	M				2B, 4M
LN12	M	M	M	B	B	M	M	B	M	3B, 6M
LN13	B	B	B	B	B	M				5B, 1M
LN14	B	B	B	B	B	B	B	M	B	8B, 1M
LN15	M	M	B	M	M	M	B	B	M	3B, 6M
LN16	M	M	M	M	M	M	M	M	M	9M
LN17	M	M	B	B	B	M	B	B	M	5B, 4M
LN18	M	B	B	B	B	B				5B, 1M
LN19	M	M	M	B	M	M	M	B	M	2B, 7M
LN20	M	M	B	B	B	B	B	B	B	7B, 2M

^aLN = Lymph Node;
^bM = malignant;
^cB = benign