Quality assurance and histopathological lesions of laboratory animals

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The extent of a health monitoring program depends on scientific requirements, but for practical and economical reasons health screening procedures must be selective (*Jonas* 1976, *Small* 1984, *National Research Council* 1991). The use of histopathology or not is one of many decisions to be considered. Naturally, gross lesions found at necropsy should be diagnosed histologically, but detection of gross lesions may depend on various factors (Table 1). Whether macroscopically normal tissues should be inspected histologically, to reveal changes demonstrable only by microscopical examination is a matter of controversy. Some laboratory animal specialists are not in favour of the use of routine histology. For them, the minimal requirements for health quality assurance of breeding colonies are fulfilled by the gross examination of tissues at necropsy, coupled with different laboratory tests (serology, bacterial cultures, and parasitology) (*FELASA Working Group on Animal Health* 1992). For others, histopathology is an essential part of health surveillance programs (*Small* 1986). The usefulness of laboratory tests rests upon their specificity, while by means of histopathology one may uncover the unexpected. Many lesions are not specific, other lesions are more characteristic, pointing at precise causes, such as diet, environmental pollutants, sanitary deficiencies, poor husbandry, etc. Thus, routine histological checks may be the only method available to alert breeders and scientists about unexpected variables and microorganisms, including microbes difficult to find by other tests. The histological changes observed are not always distinctive enough to diagnose an infection, but they will call for further examinations using more specific tests. If necessary, microorganisms may also be demonstrated in fixed tissues. Silver stains, immunohistochemical techniques, molecular hybridization, etc., have been used for the screening of microbes in tissues and animals (*Brownstein & Barthold* 1982, *Krafi et al.* 1982, *Giddens et al.* 1987, *Sundberg et al.* 1989, *Uzal et al.* 1990, *Shoji-Darkye et al.* 1991).

Table 1. Factors that may influence detection of gross lesions.

- Experience and awareness of the examinator (open mind).
- Clinical history: Disease signs, results of clinical studies, environmental assessment, etc.
- Necropsy technique.
- Type and appearence of lesions: Location, demarcation from surrounding tissues, size, colour, consistency, severity, fresh or fixed tissues, autolysis, freezing of the cadaver, etc.
- Other factors: animal species, age, and size, nutritional state, method of euthanasia, etc.

Natural infections of laboratory animals are an uncontrollable factor in experiments. Infections, however, are not the only determinant of the state of health. Animals may be defined free from numerous microorganisms, and yet be not healthy at all. Lesions may result from the expression of genetical and metabolic disturbances, ageing, numerous environmental variables, and unknown etiologies *Reznik & Reznik-Schüller* 1980, *Heine* 1982, *Lundgren et al.* 1984, *Kato & Onodera* 1984, *Van Winkle et al.* 1988, *Whittaker* 1989, *Rao-Rupanagudi et al.* 1992). The impact of spontaneous pathology on research results should not be underestimated. Experiments may be disturbed in many ways. Lesions may modify the response to test agents, or may be wrongly

attributed to them. Experimental procedures may favour the progression of changes which would develop naturally, independent of the experimental treatments. Spontaneous pathology may mimic experimental changes, or both may develop in the same tissues interfering with the interpretation of results (*Cotchin & Roe* 1967, *Kendrey & Roe* 1969, *Hinton* 1981, *Mohr & Richter-Reichelm* 1982, *Butler* 1982, *Morton et al.* 1986, *Whittaker* 1989, *Dodd* 1991, *Bucci* 1991, *Ritskes-Hoitinga* 1992). Only a few examples will be briefly mentioned. In rats lesions of chronic progressive nephrosis (CPN) are ubiquitous, and may impair renal function to the extent of causing proteinuria, hypoalbuminemia, mineral imbalances, and secondary changes in various organs. Parathyroid chief cell hyperplasia and hyperparathyroidism, mineralization of different tissues, and shortened life span are common effects of CPN in affected rats. Gross lesions of CPN, however, are not observed until the disease in advanced (*Kohn & Barthold* 1984, *Gray* 1986, *Montgomery, Jr & Seely* 1990). In long term studies decisions for using rats from one colony or other may not be based on microbial burden only but on renal lesions as well. Respiratory pathology is another ubiquitous problem of laboratory animals (*Lamb* 1975, *Feinstein & Rehbinder* 1988, *National Research Council* 1991). Since lung lesions caused by infections are prevented by excluding the etiological organisms, it is essential to test for these in screening programs. In addition, lung tissues should be controlled histologically (Fig. 1). Lesions may indicate the presence of A: Infections that escaped serological or cultural detection. B: Agents not usually included

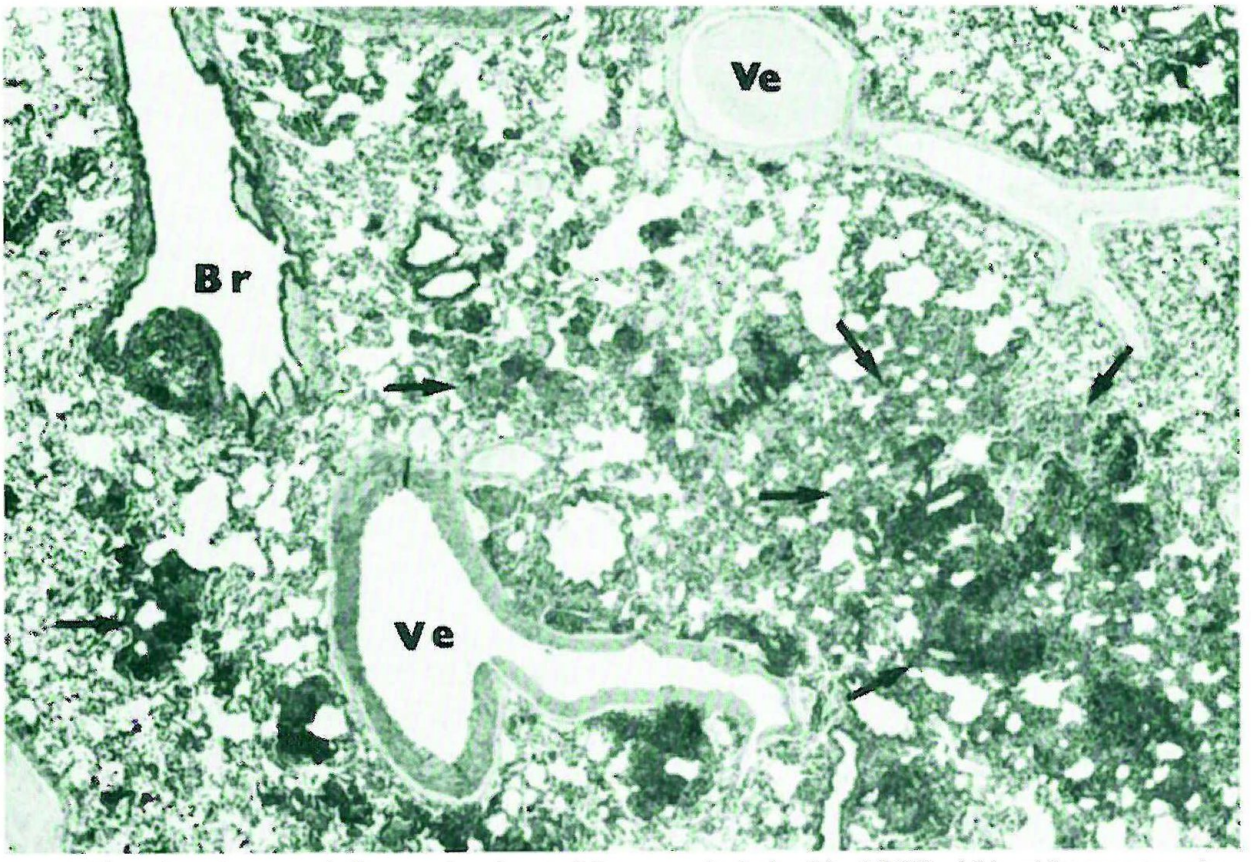

Fig. 1. Chronic pulmonary inflammation (arrows) in a seemingly healthy NZW rabbit with macroscopically normal lungs (Br: bronchus, Ve: blood vessels, Giemsa stain, × 36, necropsy number 1857). Health monitoring without histology can be misleading. Lesions, such as shown above, should not be ignored in health quality assurance programs.

in health screening programs, such as parainfluenza virus infections of guinea pigs (*Porter & Küdlacz* 1992). C: Infections for which specific tests are not yet available, like rat poxviruses (*National Research Council* 1991), or necrotizing bronchopneumonia in guinea pigs (*Feldman et al.* 1990). D: No infectious factors, and changes due to obscure or unknown causes, such as alveolar lipoproteinosis in rats, and inflammatory reactions in rabbits (*Weller* 1985, *Feinstein et al.* 1989).

In summary, the health quality of laboratory animals depends on numerous variables. Lesions is one of them, but pathology is not just the morphological changes we call lesions, it is also physiology gone wrong (*Boyd* 1961), or the expression of homeostatic mechanisms that are overstrained, even if the animals are apparently healthy. At The National Veterinary Institute, Uppsala, the health monitoring programs comprise a battery of laboratory tests, but also routine histology of major organs. That histological changes have been found in many of the animals examined, and that animal lots defined free from numerous microorganisms had lesions is hardly surprising (*Feinstein & Rehbinder* 1988, *Feinstein & Nikkilä* 1988, *Feinstein et al.* 1989), but it clearly tells that a battery of laboratory tests may not replace histology for health monitoring. Health screening programs must be selective, and histology is no exception. Decisions regarding which tissues should be inspected histologically will depend on various factors, such as scientific requirements, animal species, genetical background, sex, spontaneous pathology and natural infections of the animals examined. Additional factors, like fodder, sanitary conditions of the breeding colony or the housing, other laboratory tests included in the health screening profile, etc., should be also considered.

References

Boyd, W: Textbook of Pathology. VIIth ed., Lea & Febiger, Philadelphia 1961, *3.*

Brownstein, DG & SW Barthold: Mouse Hepatitis Virus immunofluorescence in formalin- or Bouin's fixed tissues using Trypsin digestion. Lab. Anim. Sci. 1982, *32,* 37.

Bucci, TJ: Evaluation of altered morphology. In Handbook of Toxicologic Pathology. Eds. Haschek, WA & CG Rousseaux. Academic Press, Inc., San Diego, California 1991, chapter 3.

Butler, WH: Histological control of spontaneous pathology. In Animals in Toxicological Research, Eds. Bartosek, I, A Guaitani, E Pacei. Raven Press, New York 1982, 71.

Cotchin, E & FJC Roe: Editors' preface. In Pathology of Laboratory Rats and Mice. Blackwell Scientific Publications, Oxford 1967.

Dodd, DC: The pathologist in toxicology testing and evaluation. In Handbook of Toxicologic Pathology. Eds. Haschek, WM, CG Rousseaux. Academic Press, Inc., San Diego, California 1991, chapter 2.

Feinstein, RE & C Rehbinder: Health monitoring of purpose bred rabbits in Sweden: Major findings. Scand. J. Lab. Anim. Sci. 1988, *15,* 49.

Feinstein, RE, FA Uzal & C Rehbinder: Chronic inflammatory lung lesions in rabbits free of known respiratory pathogens. Scand. J. Lab. Anim. Sci. 1988, *2,* 74.

Feinstein, RE & T Nikkilä: Occurrence of multinucleated giant cells in the appendix of clinically healthy rabbits. J. Comp. Pat. 1988, *99,* 439.

FELASA Working Group on Animal Health. Recommendations for health monitoring of mouse, rat, hamster, guinea pig and rabbit breeding colonies. Unpublished 1992.

Feldman, SH, JA Richardsson & FJ Clubb, Jr: Necrotizing viral bronchopneumonia in guinea pigs. Lab. Anim. Sci. 1990, *40,* 82.

Giddens, Jr, WE, GL Van Hoosier, Jr & LE Garlinghouse, Jr: Experimental Sendai virus infection in laboratory rats. II. Pathology and immunohistochemistry. Lab. Anim. Sci. 1987, *37,* 442.

Gray, JE: Chronic progressive nephrosis. In Urinary System, Eds. Jones, TC, U Mohr, RD Hunt. Springer-Verlag, Berlin, Heidelberg 1986, 174.

Heine, W: Importance of quality standards and quality control in small laboratory animals for toxicological research. In Animals in Toxicological Research. Eds. Bartosek, I, A Guaitani, E Pacei. Raven Press, New York 1982, 1.

Hinton, M: Kidney disease in the rabbit: A histological survey. Lab. Anim. 1981, *15,* 263.

Jonas, AM: The research and the significance of a health monitoring program. Lab. Anim. Sci. 1976, *26,* 339.

Kato, M & T Onodera: Spontaneous osteochondrosis in rats. Lab. Anim. 1984, *18,* 529.

Kendrey, G & FJC Roe: Histopathological changes in the pancreas of laboratory rats. Lab. Anim. 1969, *3,* 207.

Kohn, DF & SW Barthold: Biology and diseases of rats. In Laboratory Animal Medicine, Eds. Fox, JG, BJ Cohen, FM Loew, Academic Press, Inc., London 1984, 119.

Krafi, V, B Meyer, A Thunert, F Deerberg & S Rehm: Diagnosis of Mycoplasma pulmonis infection in rats by an indirect immunofluorescence test compared with 4 other diagnostic methods. Lab. Anim. 1982, *16,* 369.

Lamb, D: Rat lung pathology and quality of laboratory animals: The user's view. Lab. Anim. 1975, *9,* 1.

Lundgren, R, B Holmqvist, M Hesselvik & J Müntzig: Treatment of prostatitis in the rat. The Prostate 1984, *5,* 277.

Mohr, U & H-B Richter-Reichelm: The impact of spontaneous pathology on the quality of animals for toxicological studies. In Animals in Toxicological Research. Eds. Bartosek, I, A Guaitani, E Pacei. Raven Press, New York 1982, *65.*

Montgomery Jr, CA & JC Seely: Kidney. In Pathology of the Fischer Rat. Eds. Boorman, GA, SC Eustis, MR Elwell et al. Academic Press, Inc., San Diego, California 1990, 132.

Morton, D, SE Weisbrode, WE Wyder, JK Maurer & CC Capen: Histologic alterations in the testes of laboratory rabbits. Vet. Pathol. 1986, *23,* 214.

National Research Council: Health surveillance programs, Poxvirus(es) in rats. In Infectious Diseases of Mice and Rats. Eds. Comittee on Infectious Diseases of Mice and Rats. National Academy Press, New York 1991, 21 and 171.

Porter, WP & EM Kudlacz: Effects of parainfluenza virus infection in guinea pigs. Lab Animal 1992, 45.

Rao-Rupanagudi, S, R Heywood & C Gopinath: Age-related changes in thyroid structure and function in Sprague-Dawley rats. Vet. Pathol. 1992, *29,* 278.

Reznik, G & H Reznik-Schüller: Pathology of the clitoral and prepucial glands in aging F344 rats. Lab. Anim. Sci. 1980, *30,* 845.

Ritskes-Hotinga, J: Diet and nephrocalcinosis in the laboratory rat. Inaug. Dis. Utrecht Univ. 1992, 9.

Shoji-Darkye, Y, T Itoh & N Kagiyama: Pathogenesis of CAR-bacillus in rabbits, guinea pigs, syrian hamsters, and mice. Lab. Anim. Sci. 1991, *41,* 567.

Small, JD: Rodent and lagomorph health surveillance-quality assurance. In Laboratory Animal Medicine. Eds. Fox, JG, BJ Cohen, FM Loew. Academic Press, Inc., London 1984, chapter 26.

Small, JD: Decision making, detection, prevention, and control. In Viral and Mycoplasmal Infections of Laboratory Rodents. Effects on Biomedical Research. Eds. Bhatt, PN, RO Jacoby, HC Morse III, AE New. Academic Press, Inc., London 1986, 777.

Sundberg, JP, T Burnstein, LD Schultz & H Bedigian: Identification of Pneumocystis carinii in immunodeficient mice. Lab. Anim. Sci. 1989, *39,* 213.

Uzal, FA, E Rivera & RE Feinstein: Indirect immunoperoxidase and immunofluorescence staining of Bordetella bronchiseptica in formalin-fixed, paraffin embedded lung tissues of rabbits. J. Vet. Med. A 1990, *37,* 1.

Van Winkle, TJ, JE Womack, WD Barbo & TW Davis: Incidence of hydronephrosis among several production colonies of outbred Sprague-Dawley rats. Lab. Anim. Sci. 1988, *38,* 402.

Weller, W: Alveolar lipoproteinosis, rat. In Respiratory System, Jones, TC, U Mohr & RD Hunt. SpringerVerlag, Berlin, Heidelberg 1985, 171.

Whittaker, D: The importance of and difficulties encountered with diagnosis of disease in laboratory animals. Anim. Technol. 1989, *40,* 23.