THE CHOICE OF AN EXPERIMENTAL MODEL

A GUIDE FOR RESEARCH WORKERS

H. I. ROACH, J. R. SHEARER, C. ARCHER

From Southampton General Hospital and the Royal National Orthopaedic Hospital, Stanmore

Established methods of orthopaedic treatment depend on the collective experience of the profession, which changes as empirical evidence accumulates. Joint replacement, for example, which has dramatically advanced orthopaedic practice, has been largely developed by trial and error in human patients. There are, however, many orthopaedic problems which have not been solved by a clinical approach, such as osteoporosis, rheumatoid arthritis, osteoarthritis and non-union of fractures. Insights into these diseases are then sought from animal experiments.

When considering the appropriate model, orthopaedic surgeons intuitively turn to animals that are closest to the human species, but financial and ethical considerations prevent the extensive use of primates. Rabbits and mice are cheaper and more readily available, but further removed in an evolutionary sense from man. Perhaps the cheapest and most convenient source of animal material is the chick embryo, but can the results obtained with chick embryos really be related to the human situation? In this review we consider to what extent the results from animal experiments can be extrapolated to humans.

Limitations of using human materials. Ethical constraints generally limit the researcher to sampling body fluids, to follow-up studies of specific treatments and to post-mortem specimens. Significant advances have been made from such material and by careful observations of patients; an excellent overview of these is provided by the presentation given at the American Orthopaedic Association Centennial Program (*J Bone Joint Surg*

1987;69A:1257-90). Post-mortem studies, bone biopsies and tissues obtained at operations allow histological and/or biochemical descriptions, but provide little information about cellular mechanisms. Joint replacement has also enabled research to be done on diseased material, for example, the biochemical and metabolic abnormalities in osteoarthritis have been examined using femoral heads (Mankin and Lipiello 1970; Mankin et al 1971). However, only relatively advanced stages of the disease can be studied and no proper control group is available. Clinical research frequently suffers from the disadvantages that no control group is obtainable, that the sample of subjects is not representative, or that patient numbers are insufficient to reach statistical significance (Morris 1988). Therefore, the results are often inconclusive.

Animal models of diseases. In an attempt to overcome these limitations, researchers turn to animal experiments. Much of our present knowledge of fracture healing, for example, is derived from such studies. Histological descriptions of repair have helped us to understand this process and seem to apply to human fracture repair (McKibbin 1978; Brand 1979; Simmons 1985). However, attempts to study non-union in animals have been less successful, because the rate and potential for bone repair appears to be inversely related to the evolutionary scale and age of the animal (Schmitz and Hollinger 1986). Consequently non-unions are rare in animals and difficult to reproduce experimentally (Neto and Volpon 1984). Hence we do not know much about the cellular and biochemical characteristics of non-union.

The relevance of a particular animal model to a human disease rests on its ability to parallel the biological changes which characterise that disease in humans. However it is unlikely that any animal model will ever fully mimic the natural human disease. Osteoarthritis in animals, is usually caused by drastic and sudden alterations of joint function, whereas human OA develops over a period of time. Nevertheless, a lot can be learnt from animal models. Osteoarthritic lesions, for example, can be induced in dogs (Pond-Nuki model) and rabbits (Hulth, Lindberg and Telhang 1970) by immobilisation,

H. I. Roach, PhD, Senior Biochemist
J. R. Shearer, PhD, FRCS, Professor of Orthopaedics
Academic Orthopaedic Unit, Room CF86, Southampton General
Hospital, Tremona Road, Southampton SO9 4XY, England.

C. Archer, PhD, Senior Lecturer in Cell Biology
Royal National Orthopaedic Hospital, Brockley Hill, Stanmore,
Middlesex HA7 4LP, England.

Correspondence should be sent to Dr H. I. Roach.

© 1989 British Editorial Society of Bone and Joint Surgery
0301-620X/89/4195 \$2.00
J Bone Joint Surg [Br] 1989;71-B:549-53.

surgical trauma or denervation. In guinea-pigs and mice degenerative joint changes can be induced by injection of antigens into the synovium.

The characteristics of the dog or rabbit models of OA have been reviewed by Adams and Billingham (1982), Troyer (1982), Ehrlich (1985), Johnson (1986) and Schwartz (1987). Reproducibility of lesions was greatest in the immobilisation model, least consistent following denervation (Troyer 1982) and variable with the dog model (Johnson 1986). On the other hand, the arthropathy following immobilisation of the rabbit knee joint (Langenskiöld, Michelsson and Videman 1979), is thought to be caused by impaired nutrition due to lack of movement and this may be more related to prolonged limb immobilisation following orthopaedic injuries than to OA (Troyer 1982). The dog model is perhaps the closest to human OA, because dogs can develop OA naturally after accidentally torn ligaments and the characteristics of natural and experimentally induced OA seem to be similar (McDevitt and Muir 1976; McDevitt, Gilbertson and Muir 1977). Biochemical findings with the rabbit model (Ehrlich et al 1975) are consistent with those of human OA. Criticism of the guinea-pig model is that guinea-pigs or mice have no geometric analogy to the human joint. Furthermore, the cartilage in mice contains hardly any keratan sulphate, whereas in humans it increases with age.

Each model thus has its advantages and limitations of which the experimenter should be aware. Interpretation of results should not go beyond the limitations imposed by the model and it is important to formulate questions in such a way that the experiment can answer them. Thus, if the aim of a study is to examine biochemical aspects of the disease, the rabbit model might be suitable. Testing the effects of a large number of drugs may be too expensive in dogs or rabbits, but feasible in guinea-pigs or mice. On the other hand, these studies will be useless if the OA induced in guinea-pigs or mice is significantly different from human OA. Since one can never be completely certain about the relevance of results obtained from animal models, any findings from them should, as far as possible, be repeated with humans. The model merely permits the testing of many more variables.

Evolutionary closeness to man. To decide the relevance of animal experiments, clinicians frequently use evolutionary 'closeness'; results in primates are felt to be more relevant than those in rats and the latter more relevant than those in chick embryos. This is not necessarily true. To give one example, the variations in the mechanical properties of a bone are more related to its function and size than to its evolutionary position. Even the histology of the long bones of dinosaurs is remarkably similar to that of modern large mammals (Currey 1987). In other cases, selection depends on whether an animal reproduces the property under investigation. For example, it would make no sense to use dogs or rabbits for in vivo investigations on the effects of ascorbic acid deficiency

in bones, whereas guinea-pigs would be ideal. Of all mammals, only primates and the guinea-pig depend on dietary ascorbate, whereas dogs and rabbits can synthesise the vitamin (Fabro and Rinaldini 1965). The same is not true for cultures of skeletal cells or tissues which require ascorbic acid supplements (Roach, Hillier and Shearer 1985). This example illustrates two important points: first, the choice of a suitable animal depends on knowledge of the species differences with respect to the subject investigated; secondly different considerations may apply to in vitro and in vivo experiments.

The value of in vitro experiments. Because the results from studies with humans are often ambiguous, with too many variables, scientists seek simplified systems, in which one factor can be altered whilst all other variables are held constant. Culturing skeletal tissues provides such a system, but has disadvantages: in vitro systems can never give the whole truth, and the phenomena observed may be peculiar to the in vitro situation. Nevertheless, there are many instances when an understanding of basic mechanisms originated from in vitro experiments. The exact mechanism by which vitamin C deficiency causes fragile bones was established using organ culture of embryonic chick bones (Jeffrey and Martin 1966). Robison, as early as 1923, reported (after culturing chick bone rudiments) that the enzyme alkaline phosphatase played an active role in mineralisation.

Levels of organisation. It is useful to consider the following levels of organisation: subcellular; cellular; tissue; organ; organ system; whole animal (man). The mode of action of any particular agent will be at one or more of these organisational levels. Consider ascorbic acid as one example. Its role is that of a co-factor during the hydroxylation of proline to hydroxyproline during collagen synthesis. Although it acts at the subcellular level, the effects of deficiency are seen at all organisational levels: the cells secrete underhydroxylated collagen, which cannot form stable triple helices. This leads to abnormal bone matrix (tissue level) and hence fragile bones (organ level), which break easily and hence cannot fulfil their supportive role (organ system level), demonstrating the clinical signs of scurvy (whole animal and man). Here the cause of a disease was found at a subcellular level, using in vitro systems and no evidence has been found to suggest that the effects observed in chick embryos are different in humans.

This example illustrates another important point: the more fundamental the questions, the more likely it is that results obtained with, say, cultures of cells from chick embryos or mice can be extrapolated to humans. One might formulate a rule: the certainty of relevance is inversely related to the level of organisation. At the subcellular level all animals contain the same fundamental molecules (such as nucleic acids, proteins and lipids). The same types of cells (eg, osteoblasts, chondrocytes, osteoclasts) are present in chick embryos as in humans and these cell types fulfil the same functions. By contrast,

there is no guarantee that *in vivo* findings with animals extrapolate to the human situation.

Unfortunately, most experiments carried out by orthopaedic surgeons are not concerned with investigating basic mechanisms, but with testing surgical procedures or drugs, or with investigating diseases. Such experiments can only be carried out at the whole animal level and difficulties in extrapolation to humans abound.

Sometimes it is impossible to investigate basic mechanisms of a disease because of difficulties of replicating the disease in animals. To give one example, progress in the study of osteoporosis has been hampered by the lack of good animal models and by uncertainties about the aetiology of the disease. Age-related osteoporotic changes have been observed in rats (Simon 1984) and mice (Bar-Shira et al 1987), but the degree varied from bone to bone (Simon 1984) and the mechanisms may differ from those in humans. If the aetiology of osteoporosis were related to the remodelling system, as suggested by Frost (1985), then studying bone loss in ageing mice would not be very relevant to the human situation, since mice have very little Haversian remodelling. However, if osteoporotic changes primarily resulted from abnormal resorption of trabecular bone (Parfitt 1987), then mice might be suitable. Clearly, a multi-level approach is needed. Understanding the minute regulation of bone cells by local and systemic factors (cellular level) or the cell-cell and cell-matrix interactions within the bone tissue is likely to be as important as investigating risk factors and the effects of hormone replacement or calcium supplements (Peck et al 1988).

Judging the relevance of animal experiments will always depend on sound knowledge of the anatomy, biomechanics, physiology, cell biology and biochemistry of both the experimental animal and man. The researcher should be aware of species differences with respect to the subject investigated. In what follows we shall discuss some species differences in relation to several key areas.

Development of long bones. The sequence of early long bone development is almost identical in all birds and mammals until the cartilage in the centre of the diaphysis hypertrophies. Then a crucial difference is observed. In mammals, the hypertrophic cartilage calcifies prior to resorption, whereas in birds it does not. Consequently resorption of the calcified cartilage in mammals necessitates osteoclasts, whereas the uncalcified cartilage of birds can be resorbed by mononuclear phagocytes (Roach and Shearer 1989). The end result in both cases is that the cartilage of the 'anlage' is replaced by marrow, not by bone.

Mechanical properties of bone. One has to distinguish between the mechanical properties of bone as a material and the structural properties of bone as an organ.

a) *Bone material* from a wide range of species, ranging from the Galapagos tortoise to man, has been studied extensively by Currey (1987), who concluded that modern types of bone appeared at least 200 million years ago in

several different groups. Observed variations in mechanical properties arise from differences in the amount of mineralisation, the degree of porosity and Haversian remodelling (Currey 1975), which correlate with the function of the bone, the size of the animal and its life style rather than with its taxonomic position. Bones that are required to withstand impact loading, such as deer's antlers, have a low degree of mineralisation, whereas bones which need to be very stiff, such as the tympanic bulla of the fin whale, are highly mineralised (Currey 1979). Human bones have an intermediate mineral content producing intermediate values for stiffness and toughness. Cancellous bone, despite its porosity, still has 90% of the mineral content of the cortical bone from the femoral shaft (Gong, Arnold and Cohn 1964; Currey 1988), with little species variation.

b) *Whole bone strength* depends on many factors in addition to the characteristics of bone as a material, including the cross-sectional geometry and the relative amounts of trabecular and cortical bone. Again bones have adapted to their mechanical requirements and function seems to determine the variations between bones to a greater extent than taxonomy. The limb bones of small animals seem to be much stronger than they need to be (Biewener 1982), which means that the stress distribution of an applied load varies between small and large animals. Rabbit bones, for example, shatter rather than breaking cleanly.

Biomaterials. When evaluating biomaterial for use in, for instance, orthopaedic implants, two central questions have to be addressed. First, the short-term interactions between the material and the surrounding tissue, particularly in relation to integration at the implant/host interface. Secondly, the long-term effects, such as implant performance, biodegradation and possible cytotoxicity. The recent shift from biocompatible towards bioactive materials has complicated this situation even more (Ducheyne et al 1988; Kay 1988; Munting, Verhelpen and Feng 1988). In evaluating these questions, both animal experiments and *in vitro* techniques have severe limitations. Experiments with artificial joints in pigs, goats and dogs may test the mechanical suitability of implant materials, but tell us little about biocompatibility with human tissue. The latter could be investigated in cell cultures of human bone cells, but so far no culture technique consistently maintains the osteogenic phenotype in long-term culture. To increase the relevance of these cell culture experiments requires a greater understanding of the *in vitro* and environmental regulation of phenotype of connective tissue cells.

Differences in the action of effector substances. Urist, Budy and McLean (1948) showed that the reaction to oestrogen administration in growing animals was quite different from that in birds, mice, rats, guinea-pigs, rabbits or dogs. In birds and mice oestrogen inhibited bone resorption and stimulated endosteal bone formation such that the entire medulla filled with new bone. In rats

the inhibition of bone resorption at the ends of the growth plate trabeculae resulted in a 5-fold increase in the length of the metaphysis. In the other species a non-specific repression of cellular activity led to widespread degenerative changes, the formation of colloid cysts and overall stunted growth.

With regard to the main hormones controlling bone metabolism, such as PTH and vitamin D, their functions are essentially the same in birds and mammals (Urist et al 1960). However, calcitonin may not be as important in man as it is in certain animals and a marked variation in the potency of calcitonins from various animals has been observed (Albright 1979). Calcitonins from sub-mammalian vertebrates are considerably more potent than mammalian calcitonins, with salmon calcitonin having the most lasting action and potency.

Age variations. Considering ageing of the skeletal system, one must take into account two distinct, but closely related processes: ageing as perceived by gerontologists, and maturation. Both occur as a consequence of time and both involve qualitative and/or quantitative changes in the extracellular matrix. Whilst similar patterns of maturational change have been reported in animals and man (Bayliss 1986; Thonar, Bjornsson and Kuettner 1986), problems arise because of the very long life span of man. A five-year-old rabbit may be considered aged and hence this is the longest time that matrix molecules, such as collagens, will have spent in the extracellular milieu, whereas collagen molecules in humans may be present for many more years. Therefore, whilst cellular ageing may be relative to life span, changes in matrix composition may be a function of time alone. Some of these changes, such as the reduced inductive capacity of bone morphogenetic protein (Syftestad and Urist 1982), may also occur in animals, while others can only be studied in human tissues.

Conclusions. To decide on the relevance of animal experiments, one should consider whether a) the animal used is similar to humans with respect to the subject investigated (even if dissimilar in other respects); and b) whether the questions are investigated at the appropriate level, ie, whether cell, organ culture or in vivo experiments are the best to investigate the problem.

Surgeons deal with clinical problems and may be reluctant to use investigation at a level below that of the whole animal. However, a healthy scepticism should be combined with an open mind. To reject animal models or in vitro experiments as irrelevant to the human situation would be to reject part of the truth because it does not represent the full truth. Even results obtained from in vitro experiments using chick embryos may one day provide that vital missing piece in the jigsaw puzzle.

We gratefully acknowledge the help and encouragement given to us by Professor J. D. Currey of the Department of Biology, University of York, England.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES

- Adams ME, Billingham MEJ. Animal models of degenerative joint disease. *Curr Top Pathol* 1982;71:265-97.
- Albright JA. Bone: physical properties. In: Albright JA, Brand RA, eds. *The scientific basis of orthopaedics*. New York: Appleton-Centure-Crofts, 1979:135-83.
- Bar-Shira B, Coleman R, Reznick A, Steinhagen-Thiessen E, Silbermann M. Age related bone loss in vertebrae of female mice. In: Hurwitz S, Sela J, eds. *Current advances in skeletogenesis/3*. Proceedings of the 7th International Workshop on calcified tissues, Ein Gedi, Israel, March 1986. Heiliger Publ. Co, 1987:294-9.
- Bayliss MT. Proteoglycan structure in normal and osteoarthritic human cartilage. In: Kuettner KE, Schleyerbach R, Hascall VC, eds. *Articular cartilage biochemistry*. New York, Raven Press, 1986:295-310.
- Biewener AA. Bone strength in small mammals and bipedal birds do safety factors change with size? *J Exp Biol* 1982;98:289-301.
- Brand RA. Fracture healing. In: Albright JA, Brand RA, eds. *The scientific basis of orthopaedics*. New York: Appleton-Centure-Crofts: 1979:189-311.
- Currey JD. The effects of strain rate, reconstruction and mineral content on some mechanical properties of bovine bone. *J Biomech* 1975;8:81-6.
- Currey JD. Mechanical properties of bone tissues with greatly differing functions. *J Biomech* 1979;12:313-9.
- Currey JD. The evolution of the mechanical properties of amniote bone. *J Biomech* 1987;20:1035-44.
- Currey JD. The effect of porosity and mineral content on the Young's modulus of elasticity of compact bone. *J Biomech* 1988;21:131-9.
- Ducheyne P, Cuckler JM, Radin S, Healy KE, Nazar E. Bioactive calcium phosphate ceramic linings on porous metal coatings for bone ingrowth. *Transactions, 3rd World Biomaterials Congress* 1988:Vol XI:309.
- Ehrlich GE. Animal models of osteoarthritis: implication for pathogenesis and treatment. *Ration Drug Ther* 1985;19:1-6.
- Ehrlich MG, Mankin HJ, Jones H, et al. Biochemical confirmation of an experimental osteoarthritis model. *J Bone Joint Surg [Am]* 1975;57-A:392-6.
- Fabro SP, Rinaldini LM. Loss of ascorbic acid synthesis in embryonic development. *Dev Biol* 1965;11:468-88.
- Frost HM. The pathomechanics of osteoporoses. *Clin Orthop* 1985;200:198-225.
- Gong JK, Arnold JS, Cohn SH. Composition of trabecular and cortical bone. *Anat Rec* 1964;149:325-31.
- Hulth A, Lindberg L, Telhang H. Experimental osteoarthritis in rabbits: preliminary report. *Acta Orthop Scand* 1970;41:522-30.
- Jeffrey JJ, Martin GR. The role of ascorbic acid in the biosynthesis of collagen. II. Site and nature of ascorbic acid participation. *Biochim Biophys Acta* 1966;121:281-91.
- Johnson RG. Transection of the canine anterior cruciate ligament: a concise review of experience with this model of degenerative joint disease. *Exp Pathol* 1986;30:309-13.
- Kay JF. Bioactive surface coatings for hard tissue biomaterials. *Transactions, 3rd World Biomaterials Congress* 1988:Vol XI:307.
- Langenskiöld A, Michelsson J-E, Videman T. Osteoarthritis of the knee in the rabbit produced by immobilization: attempts to achieve a reproducible model for studies on pathogenesis and therapy. *Acta Orthop Scand* 1979;50:1-14.
- Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips: II. correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg [Am]* 1971;53-A:523-37.
- Mankin HJ, Lippiello L. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. *J Bone Joint Surg [Am]* 1970;52-A:424-34.
- McDevitt CA, Gilbertson E, Muir H. An experimental model of osteoarthritis; early morphological and biochemical changes. *J Bone Joint Surg [Br]* 1977;59-B:24-35.
- McDevitt CA, Muir H. Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. *J Bone Joint Surg [Br]* 1976;58-B:94-101.

- McKibbin B.** The biology of fracture healing in long bones. *J Bone Joint Surg [Br]* 1978;60-B:150-62.
- Morris RW.** A statistical study of papers in the Journal of Bone and Joint Surgery [Br] 1984. *J Bone Joint Surg [Br]* 1988;70-B:242-6.
- Munting E, Verhelpen M, Feng L.** Morphological and biomechanical study of the bone-implant interface of hydroxylapatite coated implants. *Transactions, the 3rd World Biomaterials Congress* 1988;Vol XI:308.
- Neto FLDS, Volpon JB.** Experimental nonunion in dogs. *Clin Orthop* 1984;187:260-71.
- Parfitt AM.** Trabecular bone architecture in the pathogenesis and prevention of fracture. *Am J Med* 1987;82(1B):68-72.
- Peck WA, Riggs BL, Bell NH, et al.** Research directions in osteoporosis. *Am J Med* 1988;84:275-82.
- Roach HI, Hillier K, Shearer JR.** Ascorbic acid requirements for collagen synthesis (proline hydroxylation) during long-term culture of embryonic chick femurs. *Biochim Biophys Acta* 1985;842:139-45.
- Roach HI, Shearer JR.** Cartilage resorption and endochondral bone formation during the development of long bones in chick embryos. *Bone Mineral* In print.
- Robison R.** The possible significance of hexosephosphoric esters in ossification. *Biochem J* 1923;17286-93.
- Schmitz JP, Hollinger JO.** The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop* 1986;205:299-308.
- Schwartz ER.** Animal models: a means to study the pathogenesis of osteoarthritis. *J Rheumatol* 1987;14 Spec No:101-3.
- Simon MR.** The rat as an animal model for the study of senile idiopathic osteoporosis. *Acta Anat (Basel)* 1984;119:248-50.
- Simmons DJ.** Fracture healing perspectives. *Clin Orthop* 1985;200:100-3.
- Syftestad GT, Urist MR.** Bone aging. *Clin Orthop* 1982;162:288-97.
- Thonar EJ-M, Bjornsson S, Kuettner KE.** Age-related changes in cartilage proteoglycans. In: Kuettner KE, Schleyerbach R, Hascall VC, eds. *Articular cartilage biochemistry: Workshop Conference Hoechst-Werk Albert, Wiesbaden, September/October 1985*. New York: Raven Press, 1986:273-88.
- Troyer H.** Experimental models of osteoarthritis: a review. *Seminars Arthritis Rheum* 1982;11:362-74.
- Urist MR, Budy AM, McLean FC.** Species differences in the reaction of mammalian skeleton to estrogens. *Proc Soc Exp Biol Med* 1948;68:324-6.
- Urist MR, Deutsch NM, Pomerantz G, McLean FC.** Interrelations between actions of parathyroid hormone and estrogens on bone and blood in avian species. *Am J Physiol* 1960;199:851-5.