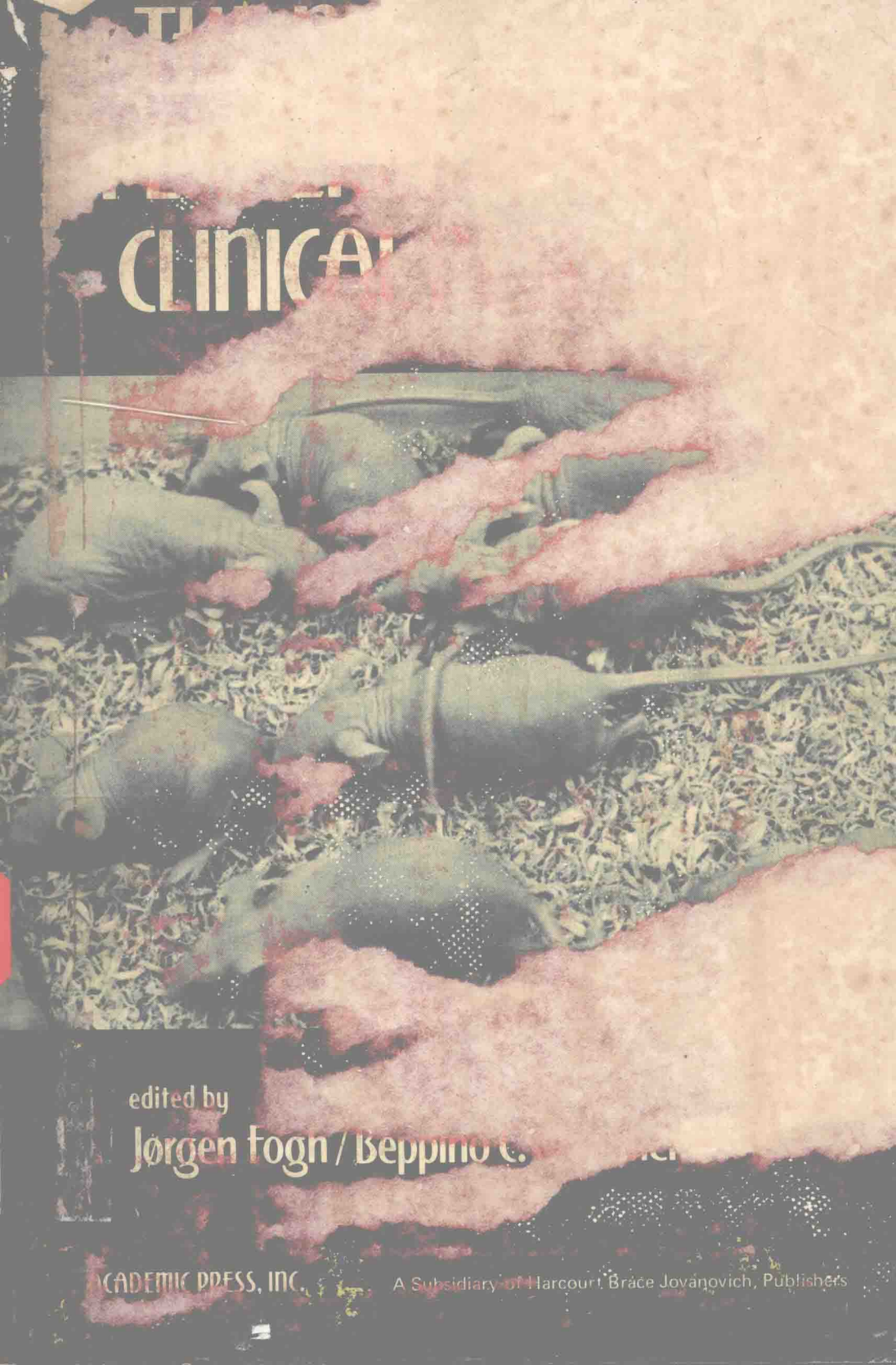
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# The Nude Mouse in Experimental and Clinical Research

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# Preface

At the time when the idea for this book was conceived, ten years had passed since the animal now known as the nude mouse was first reported. The animal's hairlessness, caused by a single autosomal recessive gene, gave this mutant its name. It grew slowly and was poorly fertile, and infections with bacteria, parasites, and viruses resulted in a very short life span. Two years after the nude was first discovered, it was learned that the animal lacked a thymus. A characteristic lack of lymphocytes in thymus-dependent areas was also reported. The nude mouse thus represented the first known occurrence of natural thymectomy and spontaneous immune deficiency in a laboratory animal.

The athymic nude mouse is not completely immunologically incompetent. While functional T lymphocytes are practically absent, the B immune system, although not normal, is present. In fact, some parts of the B system are more developed in nudes than in nonnude mice, a fact exemplified by the higher titers of IgM globulins and the larger numbers of NK lymphocytes observed in the nudes. Yet, the immunological responses of the nude were shown to be less than those of animals made immunodeficient by artificial thymectomy, and the rare value of this model for applications in immunology and cancer research readily became apparent. Fortunately, the life span of the nude mouse could be extended considerably by rather simple precautionary steps. As a result, this special animal with so many potentials was now generally available to interested laboratories. Shortly thereafter, the nude was demonstrated to accept skin transplants from other species, as well as several types of malignant human tumors and cultured tumor cells. Tumors grown in nude mice resembled the original tumors in many respects; species was consistent with the tumor cell inoculum.

Additional studies attempted to analyze the defects of the nude mouse and to explore its application in various areas of cancer research. Other efforts included studies on aging and on special hormonal and virological aspects. Genetic analysis and experimentation began to intensify, and clinical investigators soon became interested in the nude mouse, primarily for experimental cancer therapy screening. It was realized that the nude could develop infections that had previously been limited to humans, and for which animal models were either lacking or cumbersome—for example, leprosy and other tropical diseases caused by

bacteria and protozoa. Thus, researchers in a great many disciplines began to appreciate the uniqueness of the nude, with the result that important data were being collected. However, among the several observations that were still puzzling was the incidence of spontaneous tumors in this immunodeficient animal. According to an extensive interpretation of the immunosurveillance theory, it was expected that nude mice would develop many malignant tumors of all types. But in reality, only lymphomas appeared, occurring with appreciably higher frequency in nudes in comparison with their heterozygous littermates. On the other hand, when adult nudes were injected with polyoma virus, malignant tumors were produced, a finding in agreement with the immunosurveillance theory.

"The Nude Mouse in Experimental and Clinical Research" is the first major book on this subject. It seemed appropriate at this time to review the existing knowledge and the present research efforts, and to speculate on future research applications of the nude mouse. Thirty-three investigators, in addition to the two editors, have emphasized the current academic status of a broad range of subjects, and techniques are also included.

We are grateful to the many contributors who have been willing to participate in this task, and to their efforts in preparing such high-quality manuscripts. We are particularly indebted to the staff of Academic Press. The continuous dedicated efforts of Shirley DeVore, Executive Secretary, Human Tumor Cell Laboratory, Memorial Sloan–Kettering Cancer Center, have been invaluable. We present this book to the reader, convinced that investigations with the nude mouse model have reached an important stage, and we predict that future efforts in many areas of research will benefit from the knowledge collected in this volume.

Jørgen Fogh
Beppino C. Giovanella

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# 1

# The Nude Gene and Its Effects

*Carl T. Hansen*



## I. INTRODUCTION

A central theme of biomedical research is the elucidation of biological cause and effect relationships, particularly as they pertain to human health. Since these relationships are for the most part not immediately obvious, specifically designed test systems are required for their determination. Because nearly all of these test systems involve some kind of alteration of the normal functioning of the organism, it is necessary to use animal models. Animal test systems may result either from utilizing the naturally occurring genetic variation in a population of animals or from altering the natural function of the animal by artificial intervention. The naturally occurring systems are far more desirable. Most of the animal models used in biomedical research have been developed by following specific mating systems. For example, inbred strains are the product of many consecutive generations of the matings of close relatives, a process which results in a population of animals that are almost identical genetically. However, their value in the

study of cause and effect relationships is limited by the fact that strains within a given species differ only in degree. Nonetheless, studies using inbred strains have provided important insights into many areas of biomedical research.

Mutations, on the other hand, yield very specific alterations in the normal functioning of the organism. A mutation, which may be defined as a spontaneous change in a specific portion of the hereditary material, produces a precisely defined system. So defined, mutations have provided a rich source of specifically defined animal models for the study of cause and effect relationships; in many cases, they yield systems that are superior to the artificial models. However, the majority of the reported mutations are of little use in biomedical research, although all are of interest to geneticists for the insights into the nature of the hereditary material that they provide. A limiting factor in the use of mutations is that neither their appearance nor their effects can be predicted. Consequently, many mutations are maintained for many years in the hope that they will ultimately be of value in some aspect of biomedical research. There are exceptions, however. The purpose of this chapter is to provide a broad overview of a mutation reported in the mouse which, in less than a decade, has become one of the most widely distributed animal models, primarily because of one unique feature.

## II. HISTORY OF THE NUDE (*nu*)

The mouse mutant designated as "nude" and given the symbol *nu* by Flanagan (1966) is one of the rare mutants to gain immediate and broad acceptance, although the initial report did not suggest any characteristics that made this mutant valuable in many areas of research. The most unusual feature was the complete failure of the hair coat to develop. The usual situation affecting the hair coat of those mutants that have been reported, e.g., hairless (*hr*), is that the hair coat initially develops normally, but further hair growth ceases following the first molt. In this mutant, with the exception of sparse patches of hair that appear and disappear at sporadic intervals, the homozygotes are devoid of hair.

The second feature which Flanagan noted was the short life span of the homozygotes. None survived past 25 weeks of age, although no specific cause of death other than evidence of extensive liver damage could be ascribed. The short life span of mutants is not unusual since many of these mutations are deleterious. In retrospect, the short life span was probably the most significant observation.

Flanagan also established that this mutation was inherited as an autosomal recessive. The short life span of the homozygotes created difficulties in the genetic analysis. However, using special mating techniques, it was established that this gene was located on linkage group XVIII and between Rex and Trembler. The linkage information simply describes the location of the gene on the

chromosomes and is of particular interest to geneticists. The fact that this mutation involved a single gene contributed substantially to its potential value, since such a mutation could be studied and manipulated easily.

The observation reported by Pantelouris (1968) was significant in establishing the value of this gene in biomedical research. He reported that except for a small rudiment, no thymus could be found in any of the homozygotes. The importance of this observation was soon recognized, since it meant that a natural model of thymic function was available. Its appearance occurred at a critical juncture in immunology, since through extensive studies using a variety of artificially prepared models, the broad outlines of the dual nature of the immune system had been determined, as well as the central role in this response that the thymus-processed cells play.

## III. DYSGENESIS OF THE THYMUS

The thymic anlage becomes visible on day 9–10 of uterine life and incorporates ectodermal as well as endodermal contributions. A small anterior portion, the "head" of the thymus, is derived from the endoderm of the lateral and dorsal sides of branchial pouch III, and from the ectoderm of branchial arch III. The head is followed by a narrow strand that thickens from the tail of the thymic rudiment. The whole rudiment is paired. Later, the thymic rudiment moves from the cervical region to the thoracic cavity, where it grows into the definitive organ.

Pantelouris and Hair (1970) compared the development of the thymus in normal mice and in homozygous nude mice at 14–15 days of pregnancy, and 1–2 days after birth. In the 14–15-day normal fetus, determined by vaginal plugs, the two lobes of the thymus are already formed, but there is no evidence of differentiation into medullary and cortical regions. At 1–2 days after birth, the two lobes are adjacent and rest on the anterior surface of the atria and large vessels of the heart. Fibrous septa are beginning to divide the lobes into lobules, and cortical medullary zones can be distinguished. Blood vessels are more common in the cortical areas, regions which are seeded with lymphoid cells. In the homozygotes, the thymic rudiment is present at 14–15 days pregnancy, but compared to that of "normal" animals, it is much smaller and dorsoventrally flattened. At a level just below the thyroid, the thymic rudiment begins anteriorly as two separate strands, each of which has a narrow central lumen; further back, these strands become thicker and vesiculated, but always remain smaller than normal. At 1–2 days of age, a thymic rudiment is found to reach halfway between the thyroid and the heart. In section, the rudiments seem to be composed of many vesicles of canaliculi delimited by epithelial-like cells, but with no trace of