Target Organ Toxicology Series

Intestinal Toxicology

Editor

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Preface

"There was once a town where all life seemed to live in harmony with its surroundings . . .
Then a strange blight crept over the area . . .
Mysterious maladies swept the flocks of chickens; the cattle and sheep sickened and died.
Everywhere was the shadow of death. The farmers spoke of much illness in their families.
In the town, the doctors had become more and more puzzled by new kinds of sickness . . .
In the gutters under the eaves and between the shingles of the roofs, a white granular powder still showed a few patches; some weeks before, it had fallen like snow upon the roofs and the lawns, the fields and streams . . .
This town does not actually exist . . . I know of no community that has experienced all the misfortunes I describe . . ."

When author Rachel Carson described these events in her book *Silent Spring* in 1962, the story was a myth, a legend. Her critics were quick to seize on this point. Her foresight was rejected by her tunnel-visioned contemporaries. Since World War II, when the widespread use of DDT and other organic insecticides began, there has been a dramatic surge in the development of synthetic organic chemicals. Presently, there are an estimated two million recognized chemical compounds. Chemical sales are now $100 billion per year, with over 30,000 chemical substances in commerical use. To this number, a thousand new ones may be introduced each year.

Even though chemicals play an important role in protecting, prolonging, and enhancing our lives, in the past few years, many have been found to present significant health and environmental dangers. More often than not, these chemicals leak out slowly and insidiously. In some instances, the chemical insult is sudden, direct, concentrated, and awesome. These events symbolize the complex problems inherent in regulating chemical production and usage without compromising the quality of the environment and the health of workers and residents. While current regulatory agencies are examining production and utilization of chemicals, various other institutions in both the private and government sectors are concerned with the evaluation of the health effects of many of these chemicals.

This volume reviews the methods used in the assessment of chemically induced toxicity and focuses on intestinal function and toxicology. This volume represents a scientific landmark since it is an indication of the increasing awareness of the wide variety of chemicals and the level of insult that the intestinal tract is being exposed to, and of the possible intestinal damage that these exposures might cause. The intestinal wall serves both as a barrier to ingested environmental substances and, also, as a modifier of specific compounds. Alterations to these natural defense mechanisms may, in turn, allow for additional harm to other organ systems because of changes in the absorption of natural and foreign substances.

A better understanding of the basic principles of normal intestinal functions should permit greater appreciation for the unique roles of this organ in absorption and metabolism. In addition, this understanding of normal function may lead to better methods for the detection of dysfunction and malabsorption. The possible interactions of ingested substances, both in natural and metabolized forms, are rapidly increasing as are the nature and degree of intentional and unintentional contamination of ingested substances.

The purpose of this volume is to emphasize the importance of the intestines as a target organ and to provide a forum for the exchange of information among basic scientists, toxicologists, and clinicians.

It is hoped that the review of the basic sciences and the methodologies concerning the intestines and the review of the status of intestinal toxicological research will provide the groundwork for future studies in this area of target orgran toxicology. The current volume presents a reexamination of the progress within this growing research area and an elaboration of many important aspects of this new area of toxicology.

Carol M. Schiller

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Foreword

The *Target Organ Toxicology* monographs have evolved from the need for periodic review of the methods used to assess chemically induced toxicity. In each monograph, experts focus on the following areas of a particular organ system: (a) review of the morphology, physiology, biochemistry, cellular biology, and developmental aspects of the system; (b) a description of the means routinely used to assess toxicity; (c) an evaluation of the feasibility of tests used in the assessment of hazards; (d) proposals for applying recent advances in the basic sciences to the development and validation of new test procedures; (e) a description of the incidence of chemically induced human disease; and (f) an assessment of the reliability of laboratory test data extrapolation to humans and of the methods currently used to estimate human risk.

Thus, these monographs should be useful to both students and professionals of toxicology. Each provides a concise description of organ toxicity, including an up-to-date review of the biological processes represented by the target organ, a summary of how chemicals perturb these processes and alter function, and a description of methods by which such toxicity is detected in laboratory animals and humans. Attention is also directed to the identification of probable toxic chemicals and to the establishment of exposure standards that are both economically and scientifically feasible, while adequately protecting human health and the environment.

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Introduction: Intestines as a Barrier and as an Absorptive and Metabolic Organ

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This volume on intestinal toxicology is concerned with the critical appraisal of experimental methods and data obtained from research past and present and attempts to define those regions of research that should prove to be productive for our future efforts. Within these two broad objectives, the volume focuses on a number of specific areas of intestinal research. These areas of research reflect the increasing awareness and documentation of the multiple roles of the intestines, including the well-recognized and major roles of nutrient absorption and of penetrant barrier. In addition, emphasis is given to the expanding body of knowledge that relates to the intestines as a major metabolic organ involved in the synthesis and degradation of both natural and foreign substances. A further dimension is the inclusion of several discussions aimed directly at the function and dysfunction of the human absorptive processes and the possible effects of environmental toxins on these processes.

The gastrointestinal tract is assumed to be an impenetrable barrier to the uptake of intraluminal substances. Increasing experimental evidence, however, suggests that the mucosal barrier to foreign substances may be incomplete. Some macromolecules are absorbed to a degree and, although not in sufficient amounts to be of nutritional significance, may have an antigenic role or be biologically active within the organism. The development of the gastrointestinal tract during the perinatal and neonatal periods to the level of that of the adult involves a unique continuum of morphological and biochemical maturations. It is this maturation process that culminates in the highly complex organ with its many and complex functions.

A wide range of approaches and methodologies have been employed to evaluate the maturation and functions of the gastrointestinal tract. Because of its unusual morphology, the intestines can be examined *in vivo* and *in vitro* by a variety of techniques, each providing different kinds of relevant information and understanding. Utilization of sacs, loops, rings, and cells have provided ample data to reveal the importance of the gastrointestinal tract as a barrier and absorptive organ and also as a highly active major metabolic tissue. Examination of the development of

intestinal enzymes and metabolic pathways reveals an understanding of nutrient active transport and metabolism as well as metabolis n of foreign substances. The relatively large mass of this organ provides additional significance to its metabolic roles in the homeostasis of the organism as a whole.

Since ingestion is a major route of exposure to foreign substances, significant research involving the absorption and metabolism of these substances is needed. In addition, the effects of the foreign substances on the normal intestinal functions is an area of research requiring further attention. Currently, more Americans are affected by serious diseases of the gastrointestinal tract than any other system except the cardiovascular system, which provides emphasis for the increasing concern for understanding the possible environmental elements to gastrointestinal disease.

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Methods for the Analysis of Intestinal Function

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The intestinal tract is an organ of considerable complexity, comprised of numerous tissue types and subserving multiple functions. Analysis of the physiology of this system has therefore involved, and continues to require, the application of research tools from many disciplines within the sciences (63,64). The following chapter analyzes the primary function of the intestinal tract, namely the processing of orally ingested substances. Major emphasis is on determining intestinal structural integrity, absorptive function, and contractile properties responsible for transit of luminal contents.

ASSESSING THE STRUCTURAL INTEGRITY OF THE INTESTINAL TRACT

Examination of intestinal tissue by histologic techniques is useful for the detection of generalized structural alterations (58). Altered organ function may be induced, however, without changes in the microanatomy of the tissue. Lesions may be highly localized and consequently they are missed by selective sampling techniques. A noninvasive approach for monitoring the structural integrity of the mucosal lining of the intestinal tract is based on estimating gastrointestinal bleeding. The procedure entails determining occult blood in the feces by colorimetric or radioisotopic methods. The detection of fecal occult blood, an important clinical diagnostic tool (68), has application to the screening of substances for potential toxic effects on the gut and to the testing of agents with suspected or probable ulcerogenic properties such as nonsteroidal anti-inflammatory agents (44), certain food additives (38), and radiation exposure (40).

A number of procedures are available for assaying fecal occult blood. Colorimetric techniques (1,13,27,30,45) are based on the use of phenolic compounds, such as guaiac or *o*-tolidine, whose oxidation to color-emitting substances by hydrogen peroxide is catalyzed by hemoglobin. These reagents differ in their sensitivity and in their potential toxicity. Certain of these reagents are commercially available in convenient formulation for clinical use, e.g., Hemoccult, in which a slide is impregnated with guaiac. Numerous factors affect this colorimetric assay

(27). A primary variable is the peroxidase activity in the feces that originates from the diet rather than from gastrointestinal bleeding. In addition to interference from dietary factors, the results of this type of assay depend on the extent to which hemoglobin loses peroxidase activity by its metabolism in the gut lumen during transit from the site of bleeding (54). Since bleeding may be intermittent, the results will depend on the fecal sampling technique. Because of these problems, this technique is usually applied semiquantitatively.

A second approach for assessing gastrointestinal bleeding is based on quantitating radioactivity in the feces following labeling of erythrocytes with ^{59}Fe or ^{51}Cr. This procedure, which requires greater experimental intrusion than colorimetric methods, entails intravenous administration of ferrous 59-sulfate for *in vivo* labeling of erythrocytes (32,44) or of erythrocytes prelabeled with ^{51}Cr *in vitro* (39). These techniques, based on the use of radioisotopes, require the experimenter to exercise considerably greater attention to the proper housing of animals, handling of excreta, disposal of carcasses, and to other problems related to contamination and exposure. However, such approaches are more sensitive than colorimetric methods and are not subject to invalid results caused by interference from dietary sources. In addition, metabolism of hemoglobin during its transit through the gut lumen has less of an effect on a radioisotopic assay than on a colorimetric one.

Macroscopic injury to the gastrointestinal wall may also be detected by sacrificing the animal and inspecting the mucosa for lesions (50) or the serosa for perforations or adhesions (6). This technique can be made more sensitive by pretreating test animals with a nonerythrocytic vascular marker followed by its visualization on the mucosal surface or its quantitation in the gut lumen. Examples of such markers include the dye pontamine sky blue 6 BX (7) and ^{51}Cr-labeled albumin.

ANALYZING THE PROLIFERATION OF INTESTINAL MUCOSAL CELLS

A potential effect of toxic substances on the intestine is alteration of the proliferative process that occurs in the intestinal crypts. This effect may be a primary event as occurs with antineoplastic agents that impair cell division. Or, the effect may be a secondary one that occurs in response to changes in one of the many factors regulating intestinal renewal such as hormones, microorganisms, and food intake (37). Alterations in crypt cell division and migration up the villous surface may in some cases be inferred from histologic evaluation of morphology of crypt and villous cells and villous height. More definitive assessment is based on techniques of cell kinetic analysis (8). These procedures are based on pulse exposure of cells to tritiated thymidine, which is incorporated into DNA of crypt cells undergoing DNA replication. Tissue is collected at various times after thymidine exposure and thin sections processed using autoradiography. From these samples it is possible to determine the time for complete migration of newly formed cells from the crypt to the site of extrusion on the villous tip. In addition, by evaluating the fraction of mitoses in the crypt, which are labeled with ^{3}H-thymidine as a