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Current Status of Studies Concerned with Evaluation of Toxic Effects of Chemicals on the Testes

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A brief review of the history of the subject is provided. An attempt is made to define the current status of the knowledge and the reasons why our knowledge in this area of science is poor. The information gained from studies employing lower species is contrasted with information on the human male. The most commonly utilized techniques for evaluating the effects of toxic agents on the male reproductive system is briefly summarized. The growth of knowledge in physiology, biochemistry, and hormonal control of testicular function during the past ten to fifteen years is discussed; and, the specific most pertinant advances are noted. The difficulties in utilizing the human subject for toxicologic studies are pointed out and the reasons why the knowledge of the effect of toxic agents on the human testes are meager are discussed. The methodology utilized for the study of the functional integrity of the human testes and the specific parameters widely utilized for this purpose are discussed and criticized. The fact that it is extremely difficult to evaluate changes in the testicular function of men are emphasized and the extremely wide variability is pointed out. Furthermore, it is demonstrated that at this moment, we are lacking some of the most fundamental information concerning the normal function of the human testes; and, that there is a poor understanding of parameters of "normalcy". The three parameters used most commonly to define normalcy of human testes, namely, testicular histology, sperm output, and hormonal levels are discussed and the difficulties and pitfalls of these measurements are pointed out.

One of the earliest reports concerned with the effects of industrial exposure of workers to gonadotoxic substances deals with DES. Although numerous publications demonstrated that a wide spectrum of organic and inorganic molecules will induce testicular damage at below "toxic" levels no systematic, scientifically coordinated program for identification of "gonadal risk"-compounds has been promulgated by either academic, governmental or industrial organizations. This stands in contrast to programs in mutagenicity and carcinogenicity. It is of interest to note that evaluation of "new drugs" does not call for in-depth studies of their effect on the male reproductive system unless the agents are to be tested specifically as potential contraceptive agents in the human male.

In recent years the scientific community has been called upon to direct its attention towards the study of agents present in the general and industrial environments and to provide answers concerning the safety of the exposure of male gonads to the chemicals in these environments. This awakening of concern resulted from warning signs in scientific literature and from industrial episodes where a toxic substance induced sufficient damage to the gonads of the affected individuals to make them sterile and/or impotent and to force the issue into the open. The attempts to rapidly remedy years of neglect are commendable but this must be tempered with the appreciation of the fact that no systematic body of knowledge which could be used as a point of departure for such studies, particularly as it pertains to the human male, is available. The basic physiologic parameters of normalcy have still not been established for the human male (*1-7*). Techniques have not been standardized and very little effort has been directed towards developing new, more precise and more sensitive techniques.

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No formal training facilities are available and no appropriate requirements for training have been established.

The situation regarding studies in lower species is somewhat less dismal. However even here an extremely small segment of scientists trained in this discipline is engaged in studies relevant to toxicology. This may possibly be due to the lack of interest resulting from lack of funds for support of toxicologic studies. It has been recently stated that "the toxic effects of drugs and environmental chemicals on human reproduction have become a major health concern; incidence of chemically-induced germ cell damage and sterility appear to be on the increase" (*8*). Unquestionably one of the approaches to counteract this increase is to acquire more adequate basic knowledge and to apply this knowledge to the problems at hand. The other avenue would be to cease production of all chemicals and to curtail the use of chemical sources of energy, an essentially unacceptable and probably unenforceable alternative.

Most of our knowledge concerning the toxic effect of various classes of chemical compounds on the testis is a by-product of studies directed to answer questions in other areas of science. In most instances the studies were directed towards synthesis of carcinogenic chemicals for development of models to study cancer, mutagenic agents to study the mechanisms of mutagenicity, insecticides, herbicides, pesticides, antibiotics or anticancer agents.

Some of the earliest reports on chemically induced testicular damage resulted from studies of agents like nitrogen mustard, various other alkylating agents and antimetabolites (*9-12*). While a great deal of effort has been devoted to relate the structure of these compounds to their effects as carcinogens, anticancer agents, alkylating agents, etc., very little effort has been directed towards the study of the relationship between their chemical characteristics and gonadal effects, except for studies related to mutagenicity of these compounds and their effects on mitotic activity (*12, 13*) or studies related to the development of male contraceptives (*14*). While on one hand classes of chemicals as diverse as nitrofurans, cadmium, or fluoroacetamide were shown to affect spermatogenesis directly, within each class of these compounds different derivatives were shown to have markedly varying effects. Up to date no serious attempt has been directed towards elucidation of the biochemical mechanisms responsible for the effects of a given chemical moiety on spermatogenesis and the absence of such effect when only slight modification of the compound's structure is made. Even now, in most instances, studies of effects of chemical agents on the testes of experimental animals are limited to relatively crude evaluation of testicular function; assessment of organ weights, subjective evaluation of the histologic picture of the testes, evaluation of sperm output and fertility, and measurement of hormone levels in blood. In most instances, the effects of the various chemicals on the testes has been shown to be cytotoxic in nature (in other words all or certain type of germinal cells are destroyed after exposure of the animal to the chemical), or to produce lethal mutations resulting in formation of spermatozoa which upon fertilization cause zygotes which are unable to proceed with further embryonal development; the gestation terminating with resorption of the embryos. In the few instances where biochemical parameters (e.g., enzyme activities) were assessed, the observed changes (decrease in activity) was usually associated with morphologically demonstrable destruction of a specific population of germ cells. Such studies do not allow any conclusions concerning the specific effect of the chemical on the enzyme system since the changes in enzymic activities could be due to the destruction of the cell by the chemical rather than to an effect of the chemical on the enzyme.

The advances made in our knowledge related to biochemical mechanisms concerned with testicular function have been remarkable in the past decade. Probably the most important discoveries are concerned with studies demonstrating the importance of Sertoli cells in the response of the seminiferous epithelium to FSH and testosterone (*15*), with the evidence of the pivotal role of testosterone in spermatogenesis (*16*), and the studies on blood-testis barrier (*17*).

The work leading to the demonstration that the Sertoli cell is the target for FSH was made possible by the development of techniques for isolation and establishment of cultures of pure Sertoli cells (*18, 19*). This led to demonstration (*20, 21*) that FSH receptors are present exclusively in the Sertoli cell membranes, that Sertoli cells respond to FSH with activation of adenyl cyclase and formation of cAMP, and that the Sertoli cells produce specific proteins in response to FSH or testosterone stimulations: ABP and the Sertoli cell factor (a protein probably similar or identical to the "inhibin"). A considerable body of information has accumulated concerning activation of phosphorylization processes and synthesis of RNA (*22*). Recently, an acceptor site for the testosterone-receptor complex was demonstrated on the chromatin of the Sertoli cells (*23*). Considerable information has been accumulated in respect to the understanding of spontaneous and drug induced unscheduled DNA synthesis in the germ cells (*8*). Hypotheses dealing with the molecular mechanisms

concerned with hormonal control of spermatogenesis have been proposed (*15, 24*). Most of the work, however, reflects findings in a limited number of species and very little information in man.

Elegant studies on the blood-testis barrier in animals demonstrated differential permeability of this barrier to various biological substances and drugs (*9*). Unfortunately only limited data are available concerning the presence and behavior of the blood-testis barrier in man.

While limited attempts to utilize this knowledge to the study of the effects of toxic chemicals on testis of lower species were made very little if any work has been conducted at this level with human testes. First, most of the basic information available for the testes of lower species is only now being investigated in the human. These investigations are conducted in very few laboratories. Second, the evaluation of the effects of noxious agents on the human male reproductive system is difficult. The reasons for these difficulties are numerous: (1) in most instances only retrospective studies can be conducted; thus, one has to depend on epidemiologic rather than experimental approach. (2) The sampling of the affected population cannot be ideal because of moral, ethical, legal, and circumstantial considerations which are especially magnified because of the nature of the target tissue. (3) The parameters used for evaluation are by necessity primitive and of low sensitivity. The data are difficult to interpret.

The three most accessible and most commonly employed methods for evaluation of testicular function in man are: the determination of sperm density in the ejaculate; determination of plasma hormone (testosterone, LH and FSH) levels; and subjective assessment of the microscopic appearance of the testicular tissue (*25*). These methods are extremely simple (except for determination of the hormone levels), but frequently are poorly performed and inappropriately interpreted. Many studies reported in the literature were conducted in laboratories with expertise other than male reproductive biology. Thus the generated data are imprecise, incomplete or diffieult to interpret.

Evaluation of the sperm output in the human male is fraught with numerous pitfalls, commencing with the technique of procurement of the specimen and ending with the attempt of the investigator to place the results within the framework of normalcy for the specific population of which the individual is a member (*1-5*). In addition one has to deal with the question of "normal" variation in sperm output of the same individual sampled at different times (*26*). Sporadic drops in sperm output may occur secondary to uncontrolled factors e.g. systemic or localized viral or bacterial infections, various forms of stress, allergic reactions, etc. (*27, 28*). Also, seasonal changes in sperm output have been reported to occur (*23*). Furthermore, frequency of ejaculations during weeks preceding the collection of the specimen alters sperm output (*29*). Obviously azoospermia or extremely severe oligospermia strongly suggests an important deviation of the sperm output from normal, particularly if this state persists for months. However, detection of azoospermia is of little value in investigation of partial or "mild" effects.

The sperm output data may be used with considerable effectiveness when sufficiently large populations of exposed individuals are examined and the pattern of the frequency distributions of the sperm counts is compared with that found in "normal" populations. Unfortunately in spite of a number of attempts to estimate the frequency distribution of sperm counts in populations of "normal" individuals we still do not have acceptable data (*3*). Furthermore the influence of ethnic background, geographic location, and socioeconomic factors on the sperm output is not known.

Testicular biopsies are commonly used in evaluation of spermatogenic function in testes of men. However, due to faulty procurement techniques and inadequate histologic processing the preparations are often uninterpretable. Also since the interpretation is frequently carried out by individuals not trained specifically in the area of pathomorphology of the testes, erroneous conclusions are made. Seldom are appropriate quantitative techniques employed.

It is unfortunate that the few studies conducted in men exposed to substantial amounts of toxic chemicals did not include adequate histologic evaluation of the testes or quantitative analysis of spermatogenesis. It should be noted that considerable information concerning the quantitative aspects of the spermatogenic response to ionizing radiation in man has been obtained under more optimal conditions (*30, 31*). A number of investigators demonstrated the effectiveness of careful histologic techniques and their value in quantitative evaluation of human spermatogenesis (*32-34*). Undoubtedly these techniques should be employed in studies dealing with the effects of environmental and industrial toxic agents on spermatogenesis. Application of these techniques to evaluation of testicular tissue from men with oligospermia, which in some instances indeed could have resulted from unsuspected exposure to environmental or industrial pollutants, demonstrated clearly their utility and importance (*6, 34, 35*).

The measurement of hormone levels in circula-

tion is useful but again it has numerous limitations, particularly when single samples are tested (*25*). Such measurements are of questionable value for determination of less than "gross" changes. Even multiple sample techniques provide only limited information. Elevated plasma FSH levels are frequently found in men with damage to the seminiferous epithelium; however, serious disturbances in spermatogenesis have been observed in men with "normal" FSH levels (*36*). The normal range for plasma testosterone levels is extremely wide. Detection of levels within this range is commonly interpreted as indicative of normal Leydig cell function. Function tests (e.g., HCG stimulation) demonstrate that this may not be the case. The Leydig cells "compensate" and produce sufficient amounts of testosterone to result in "normal" circulating levels in spite of intrinsic cell damage (*37*). This point is of particular importance when toxic chemicals, which may have a direct deleterious effect on the Leydig cells, are evaluated.

In summary, considerable information has been acquired in the past three decades on physiology and biochemistry of testicular function in animals. However, no systematic studies have been supported to adequately elucidate the effects of toxins and environmental factors on these functions. In man even less has been accomplished probably because of the lack of basic knowledge, difficulties in the experimental design and lack of adequate experimental techniques.

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