THE ROLE OF LABORATORY ANIMAL STUDIES IN ESTIMATING CARCINOGENIC RISKS FOR MAN

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It is a pleasure and an honor to have this opportunity to speak to you all this morning and to talk about one of my favorite topics -- or in fact better, a number of my favorite topics all at once.

These are three in number. They include: first, the universality of biologic processes; second, the social usefulness of biomedical research; and last, the exploitation of opportunities for effective preventive medicine.

The universality of biological processes articulates the concepts that molecular, cellular, tissue and organ functions are strikingly similar from one animal species to another. Processes from Na and K transport and ion regulation, to energy metabolism and DNA replication vary little in the aggregate as one moves along the phylogenetic ladder. The classic work on the transmission of neural impulses in the squid axon is directly relevant to man. Extensive renal function studies in fish, rodents, and dogs set the basis for our current understanding of renal function and the treatment of hypertension in man.

The simple, practical implications of this concept are at the core of the topic for this meeting.

In laboratory animals we have a surrogate for human beings. It is possible to test those chemicals to which man is or will be exposed in laboratory animals, and if the tests indicate a hazard, to control the use of that chemical to avoid or minimize human exposure. The consequence of this sequence of events is to avoid or minimize human disease. This

sounds simple and logical, but all of us in this room are aware of the immense practical problems associated with this approach. I am convinced, however, that the problems are solvable and that this approach is feasible and that it must be taken.

Let us look at some of the scientific and technical problems:

Scientific and Technical Problems:

Critical: To what extent do biological responses in laboratory animals to chemicals predict qualitatively and quantitatively responses in man?

Secondary: What are best strains, species, experimental conditions, etc.?

What will be the role of new test systems based on mutation production, interference with DNA, cellular transformation, etc.?

How are the enormous amounts of data to be processed, distributed, analyzed, integrated and understood?

There are additionally a variety of managerial, economic and social problems which I shall leave to others. It is appropriate that I focus on the critical scientific problem.

Many scientists expert in the care, feeding, and understanding of rodents and their response to carcinogens and non-carcinogens appear to be reluctant to apply their knowledge to predict what will happen when man is exposed to these chemicals. This, I think, is understandable. Let me

explain. A scientist likes to think up an idea or propose a hypothesis, then design and execute an experiment that can rigorously test that hypothesis. And only at that point does the scientist publicly explain to the other scientists the nature of the hypothesis and the results of the experiment. In projecting the results of carcinogenicity tests from laboratory animals to hypothesize what will happen to man, the scientist is denied the opportunity to test his "idea." The idea or hypothesis is, of course, the prediction that a chemical will or will not produce some estimated probability of cancer in man given a certain level of exposure.

The laboratory scientist, accustomed to being able to close the circle from hypothesis, to test, to acceptance or rejection, and new hypothesis generation, is uncomfortable when lawyers, economists, and politicians take the hypothesis and use it in a system in which the circle cannot be closed and in which the answer often cannot ever be known.

There are now instances in which the circle has been closed -- in which, irrespective of the sequence of events, we have carcinogenicity data from laboratory animals and from human exposure. I believe these data are among the most precious in the biomedical community because of the human suffering involved in their acquisition and because of the possibility of the human suffering that their intelligent use can prevent.

These examples in which laboratory animals and human populations have been exposed to the same carcinogenic chemicals supply us with an opportunity to look in a scientific manner at the question: To what extent do biological responses in laboratory animals to chemicals predict responses in man?

Bernard Altschuler of New York University in an unpublished manuscript reviewed 337 materials reported in the first 13 IARC Monographs in an attempt to answer the question: "How likely is a demonstrated animal carcinogen to be a human carcinogen." Among the 337 materials he found relevant data on both animal and man on 81 "positives" in animal studies. Of these he classified 0 as "negative" in man and 22 as having "definite," 59 as having "less definite" evidence of carcinogenicity in humans. In animals he also found one material was negative and one was "less definite" which were positive in man. Using this classification, Dr. Schneiderman has calculated that this gives, as the sensitivity of the animal screen: (1 - percent false negatives) of 1 - 1/81 = .9756, or almost 98%. For the "specificity" (1 - percent false positives) his numbers lead to 1 - 0/21 = 100%.

In the first 16 volumes Tomatis, Wilburn and Hutt report that:

"25 chemicals or industrial processes are associated with, or are strongly suspected to be, associated with the occurrence of cancer in man." (Table 1)

The five industrial processes for which the chemical identity of the causative agent is not known are shown in Table 2.

There is strong evidence that one or more materials involved in each of these five processes is carcinogenic in experimental animals.

The other 20 are single identified chemicals.

Arsenic, which is associated with skin cancer when ingested in drinking water and lung cancer after occupational exposure, has not been shown to be carcinogenic in laboratory animals. Reported laboratory

animal studies on benzene, chloramphenical, oxymethalone and phenacetin have not been adequate. The other 15 compounds are carcinogenic in both laboratory animals and man.

These data lead the working party which recently revised the preamble to the following conclusion. I shall quote from my notes and Dr. Schneiderman's and Dr. Hoel's of the final agreed upon concepts. The final published language may well differ, not in context, but by virtue of its more elegant syntax and phraseology.

> "Evidence compiled from the first 16 monographs shows that of about 25 chemicals, chemical mixtures and industrial processes now generally accepted as causing cancer in man, all but two (arsenic and possibly benzene) which have been tested appropriately have each been shown to produce cancer in at least one animal species. For several of them, e.g., 4-aminobiphenyl, diethyl-stilboestrol, mustard gas, vinyl chloride and aflatoxins, evidence of carcinogenicity in experimental animals has preceded evidence from epidemiological studies or case reports.
>
> A number of chemicals, not known to be carcinogenic for man, have been shown to produce tumors in animals and these can be assigned to two groups: (1) chemicals which have been unequivocally shown to produce malignant neoplasms (often considered as "strong evidence" chemicals) and (2) others in which the evidence of carcinogenicity is based solely on the appearance of such neoplastic lesions as lung adenomas and hepatomas in mice (often considered as "weak evidence" chemicals). In the "strong evidence" group, where the chemical has been studied in both animals and in man, there is no case in which there is sufficient epidemiological evidence to demonstrate that the substance is not carcinogenic to man. For all "strong evidence" chemicals, it is thus reasonable to treat the agent as if it were carcinogenic for man. In the "weak evidence" group (where the evidence for human carcinogenicity is also weaker [cf DDT, isoniazid, etc.]), while such chemicals may indeed be carcinogenic for man, more experimental and epidemiological work is required. Adequate epidemiological studies have not been done for most chemicals in both these categories -- often because the necessary study situations do not exist, even though human exposure may have occurred in many instances."

Thus, there is clear historical evidence that if there is strong evidence that a chemical is carcinogenic in appropriate laboratory animal test systems, it must be treated as if it were carcinogenic in man.

A number of very recent studies make this conclusion more secure. Rossman and Troll have demonstrated that in unicellular systems arsenic inhibits DNA repair. Quite possibly then arsenic may be a co-carcinogen that facilitates the action of carcinogens. This would explain the epidemiological finding of an association between arsenic exposure and cancer and strengthen the concept that exposure to arsenic should be avoided.

With regard to benzene, both Nelson's group at New York University and Maltoni's group at Bologne have new, unpublished data strongly implicating benzene as a carcinogen in rodents.

There is also new evidence as yet unpublished that phenacetin is carcinogenic in laboratory animals (Odashima).

The working group has suggested that 4-aminobiphenyl, DES, mustard gas, vinyl chloride and aflatoxins were shown to be carcinogenic in laboratory animals prior to evidence that they were carcinogenic in man.

I believe that two more could be added to that list. Consider first bischloromethyl ether (BCME). Informal reports of an increased incidence of lung cancer in the Bridesburg plant of Rohm and Haas occurred from possibly as early as 1956 on. In 1962 Rohm and Haas sent Sloan Kettering Institute a list of 102 chemicals handled in the suspicious area When asked about this later (in the mid 1970s) Sloan Kettering noted that: "We did receive a letter about an enclosed list of

compounds. We had no knowledge of the definite reason we were being asked to look at the list." They reported "no compellingly suspicious" carcinogens.

In 1964 Rohm and Haas contacted Dr. Norton Nelson at New York University Medical Center concerning the possibility that one of the chemicals was a lung carcinogen. Drs. van Duuren and Nelson, after looking at the plant and the list of chemicals, identified BCME -CME as likely to be direct acting carcinogens and designed a series of experiments to test that possibility. After negotiation, however, Rohm and Haas and New York University could not agree on the terms of a contract to test the compounds. Later, with NIH money, New York University identified BCME as a potent carcinogen.

Rohm and Haas went to Hazelton Laboratories, a private industrial research laboratory. In susceptible mice BCME exposure tripled the normal incidence of pulmonary adenomas in six months. This was reported to Rohm and Haas by Hazelton in 1967, and the open kettle process in which BCME was generated was converted to a closed process in 1968.

Thus, a general suspicion led to animal experiments that precisely pinpointed the chemical, BCME, one of over a hundred chemicals, and the disease, oat cell carcinoma, that occurred in both exposed experimental rats and exposed workers.

I would submit that animal data predicted that the medical use of estrogens would cause an increase in cancer. In 1974 an IARC group noted that "as stated in the general introduction (see p. 11), 'at the

present time no attempt can be made to interpret the animal data directly in terms of human risk since no objective criteria are available to do so.' There is, therefore, no substitute for direct observation in the human being, although the animal experimentation provides important clues as to where one should look for human risks."

In the subsequent years, of course, evidence linking conjugate estrogen use with endometrial cancer in women has been forthcoming.

These examples convince me that, like it or not, we are in the midst of a revolution in the way the scientific community identifies carcinogens. In the 1770s Sir P. Pott used epidemiological tools to identify carcinogens. In the 1970s such scientists as Nelson, van Duuren, Maltoni, and many others used laboratory animal studies to identify chemicals later shown to be carcinogens in man. We have learned a lot in 200 years.

The scientific community -- and the downstream lawyers and politicians -- are beginning to believe that animal tests can and do predict for carcinogenicity in man.

If this qualitative agreement is accepted, the next question concerns quantitative aspects of the broad area of risk assessment. There are two aspects to this. What will be (or is) the exposure level in the human population? This important question I will not comment on but clearly the answers are needed and fortunately usually it is straightforward to obtain the answers. The second question -- relevant to this meeting is: What is the quantitative relationship between the amount of a carcinogen that causes cancer in laboratory animals and in the human population?

This problem is particularly difficult. Dose response data can be easily, if expensively, obtained in laboratory animals. Similar data are almost non-existent in human populations. The only systematic attempt to estimate and compare dose-response relationships is found in the USA NAS/NRC report on Pest Control, Vol. 1, Contemporary Pest Control Practices and Prospects (1975). To quote:

> "The finding that most compounds known to cause human cancer are demonstrably carcinogenic to laboratory animals is a qualitative one. For risk estimation it is necessary to have some quantitative estimate regarding the sensitivity of man relative to that of laboratory animals. . . For the few carcinogens for which comparisons may be undertaken, the total induced incidence in man and the intensity of exposure are usually very poorly known, and the duration and conditions of exposure are often not comparable in the available studies on animals. Nevertheless, in order to bring together some of the data and to encourage more adequate comparative studies, the Panel reviewed the available data for the small number of carcinogens for which human exposure and induced incidence may be at least roughly estimated. These carcinogens are benzidine, chlornaphazine, DES, aflatoxin, vinyl chloride, and cigarette smoke. . . Dose is expressed as the total amount of carcinogen ingested, injected, or inhaled per kilogram of body weight. Where several animal tests involving prolonged exposure are published, the ones indicating the highest sensitivity for each species have been chosen. In the case of DES, a single dose experiment on newborn female mice is also included since it more closely simulates the conditions under which prenatal exposure is known to cause cancer in women."

> "The carcinogens for which comparisons can be made are those already known to affect humans. In generalizing to other compounds, this selection may impose a bias, exaggerating the sensitivity of man relative to laboratory test systems. Other factors may introduce an opposite bias. For two carcinogens, vinyl chloride and DES, observations on man are for considerably less than a full lifetime so that the reported incidence may be a serious underestimate of the eventual total. Also, these two compounds and aflatoxin are known as human carcinogens because they are associated with types of cancer that are otherwise rare. If these carcinogens also induce more common types of cancer, even at much higher frequency, this could go undetected, again giving rise to an underestimate of their overall carcinogenicity to man."

"The limited conclusion that emerges from the comparisons. . . (in Table 3) is that if the data from the most sensitive published test on animals are used to predict lifetime human incidence on a dose per body weight basis, the result seems approximately correct for benzidine, chlornaphazine, and cigarette smoking. For aflatoxin, the predicted human incidence is about ten times greater than estimated from existing epidemiologic studies; for vinyl chloride it is about 500 times higher. For DES, the human incidence predicted from the result of a single dose administered to newborn female mice is about 50 times higher than that estimated from studies of adenocarcinomas in daughters of women given DES during pregnancy. Thus, as a working hypothesis, in the absence of countervailing evidence for the specific agent in question, it appears reasonable to assume that the lifetime cancer incidence induced by chronic exposure in man can be approximated by the lifetime incidence induced by similar exposure in laboratory animals at the same total dose per body weight."

The recent working group concluded; and again I shall use Dr. Hoel's and my notes:

"A quantitative correlation between laboratory animal data and human data is less clear, even for chemicals that induce a range of malignant neoplasms in animals, because of the limited human data. However, general biological considerations and scanty human information that is available suggest that a quantitative correlation may exist, at least within a particular category of chemicals (e.g., perhaps, the mutagens). Evaluation of quantitative correlations in terms of say mg/kg/day should take account of physiological, pharmacological and toxicological differences between test animals and humans."

It is very important that IARC follow very closely future developments in this area and in addition let me emphasize the need to encourage research which will permit more and better comparisons to be made between results in laboratory animals and in exposed human populations.

To recapitulate briefly: (1) There is very considerable evidence that chemicals which are carcinogenic in laboratory animals are carcinogenic in

human populations, if appropriate studies can be performed -- qualitative predictability. (2) There is tentative evidence that there can be a quantitative relationship between the amount of a chemical that is carcinogenic in animals and that which is carcinogenic in man.

This information may now be used to prevent cancer in man.

Let me suggest a general outline of how society might proceed to do this. I start with the premise that human exposure to carcinogens is inevitable.

As long as we burn fossil fuels, some human exposure to benz(a)pyrene and similar polycyclic aromatic hydrocarbons will occur.

Aflatoxin exposure seems inevitable.

Nitrosamine exposure seems inevitable.

Exposure to asbestos seems inevitable. And so forth.

Society should attempt to keep such exposures at a minimum, and to do this rationally we need to estimate the risk to the human population from each of these exposures to guide the intensity of our efforts to reduce exposure.

But what about chemicals to which exposure is not inevitable? A certain fraction of these chemicals appears to be carcinogenic, yet I am unsure of the societal need for them. "Need" is a word that seems to have a variety of shades of meaning. Does a pre-teenager need an artificially sweetened soft drink? Indeed, does a teenage diabetic need such a drink? Does a 60 year old man need a red cherry in his "Manhattan" or a six year old girl need one in her "Shirley Temple?"

"Need" is often defined and indeed promoted by those who produce, incorporate or sell products that may be carcinogenic. So let us be careful about

the interpretation of need.

The societal need must be balanced against the estimated risks to society, for this is the only way any rational decision may be made. With the plethora of synthetic organic chemicals in use today, it will be a major task to develop the data base to allow risk estimations. But it must be done, for how can society intelligently regulate chemicals which pose human benefits and human risks without having an estimate of the risk?

The social usefulness of biomedical research is clearly illustrated by our demonstrable ability to predict which chemicals will be carcinogenic in man and our beginning ability to quantitate these risks. Much of that research has led to an understanding of the process by which chemicals cause cancer in mammals. We now understand much more about initiating factors and promoting factors about processes and pathways of metabolic toxication-detoxication mechanisms and about the role of mutations and DNA repair processes. It is this understanding that strengthens my confidence in our ability to make reliable predictions.

The implications for preventive medicine are obvious. The causes of half or more cancers in the human population are unknown. To the extent that environmental chemicals are involved in the cause of human cancer, identifying and controlling carcinogenic chemicals could have a major impact.

And that is up to you.

Table 1

CHEMICALS CONSIDERED IN VOLS. 1-16 FOR WHICH EVIDENCE OF CARCINOGENICITY TO MAN EXISTS

Aflatoxins
4-Aminobiphenyl
Arsenic compounds
Asbestos
Auramine (manufacture of)
Benzene
Benzidine
Bis(chloromethyl)ether
Cadmium oxide
Chloramphenicol?
Chromium (chromate producing industries)
Cyclophosphamide
Diethylstilboestrol
Haematite (mining)
Isopropyl oil
Melphalan
Mustard gas
2-Naphthylamine
Nickel (nickel refining)
N,N-Bis(2-chloroethyl)-2-naphthylamine
Oxymetholone?
Phenacetin
Phenytoin
Soot, tars & Oils (PAH's)
Vinyl chloride

? indicates a strong suspicion

Table 2

INDUSTRIAL PROCESSES FOR WHICH
THE CHEMICAL IDENTITY OF THE CAUSATIVE AGENT IS NOT KNOWN

Process	Target organ in humans
Manufacture of auramine	Bladder
Chromate producing industries	Lung
Exposure to cadmium (possibly cadmium oxide)	Prostate ?Lung
Haematite mining	Lung
Nickel refining	Nasal cavity, lung

Table 3

	Predicted Human Incidence based on Most Sensitive Animal Species is
Benzidine	the same as
Chlornaphazine	the same as
Cigarette Smoke	the same as
Aflatoxin B_1	10 x greater than
DES	50 x greater than[x]
Vinyl Chloride	500 x greater than [x]

existing epidemiological studies would suggest.

[x]population still at risk