TESTING FOR DIETARY CARCINOGENICITY

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Dr William J. Darby[1], Chairman of the Food Protection Committee of the National Research Council, recently pointed out that " it is necessary to have some concept of use, regularity of appearance in the diet, and so forth, in order to appraise the problem or in order to appraise, or judge, safety associated with the use of food additives". In other words, we must be sure that food additives in the concentrations used are not toxic and that they will not contribute to, or produce, cancer.

Pharmacologists realize that, with thoughtful planning, the determination of the pharmacodynamic action and toxicity of a new additive represents a straightforward problem. How to determine the possible carcinogenic potency of a compound is, however, another matter. The fact is that we do not have dependable straightforward methods of approach; we do not even know what goes wrong in an organ, in a tissue or in a cell, that sets off the trigger causing cancer.

The first question to settle is how one should start. Should we agree with Dr Emerson Day[2] of the Sloan-Kettering Institute for Cancer Research, who stated before a Subcommittee on Health and Science[1] that " to determine causes of cancer in man and to bring about control we believe that we must study data which originate in man " and who continued, " all the animal work in the world, from my point of view, does not help us understand how to control cancer in man unless there are counterparts in man's experience "? Or shall we agree with Dr Morton L. Levin[3], director of the New York State Department of Health, who stated that " our first line of defence in studying the possible carcinogenic effect of such substances as food additives should be animal experimentation, but we cannot rely exclusively on animal experimentation for the possible discovery of such effects "? Supplementing animal studies, Dr Levin described four epidemiological methods which may be used to trace the cause of cancer. He considered his third method, " the study of special populations", particularly suitable. By special populations, he meant people engaged in the manufacture of a particular chemical. Or should we exert our primary influence as recommended by Dr Herbert E. Carter[4] of the University of Illinois who is more optimistic and who believes that progress is likely to come from the study of a single cell? He believes that " there will be many ways in which it may be possible to determine how a cell reacts to the presence of a carcinogen long before the final demonstration of the irreversible transformation into a cancer cell itself".

It has been stated repeatedly that most of what we have learned of human cancer was discovered in man. This is correct and perhaps, for this reason, we should confine our studies to man. In this case, the simplest method of arriving at an answer would be to divide our population into various groups —experimental and controls—to feed them various suspected carcinogens and then to sit back and wait for 20 to 30 or more years for some type of cancer to develop. Obviously, this is not a method that we would choose; however, judging from the prevalence of human cancer, are we not actually doing this in an uncontrolled way?

For the past years, many of us have undoubtedly absorbed—perhaps by inhalation, by ingestion or possibly by absorption through the skin—certain chemicals which are responsible for at least some cancers with which many are afflicted today. This certainly applies to some of the industrial population or to people living in highly industrialized areas.

The causes of about 99 per cent of human cancers are not known. Exposures to β-naphthylamine and xenylamine (*p*-aminobiphenyl) have in well-documented instances led to bladder cancer, and it seems entirely possible that other, not-yet-identified, carcinogens are in use. Certainly, cancer is a common affliction, and our records show that one of each four persons will suffer from this disease sometime during his life.

Epidemiological studies have pin.pointed certain chemicals as carcinogenic agents. But, since the first appearance of cancer usually follows a latent period of 15, or 20, or more years, we must be lagging 15 or more years behind the responsible contact if we place much reliance on such methods. To those who attach undue significance to epidemiological studies, I should like to say that these investigations have detected the causes of considerably less than 1 per cent of the human cancers. It is true, as Dr Levin pointed out, that most of what we know about chemical causes of cancer originally was discovered by observation of humans. But are we committed to this method exclusively? Most of what we have learned about lead, mercury, arsenic and certain other poisons was also discovered in man. But certainly, for the effects of new industrial materials, we do not continue to look to man as the experimental animal of choice. Everyone in the fields of occupational medicine, industrial hygiene and toxicology realizes that pre-testing of chemical compounds in experimental animals has prevented untold intoxications in man during the past 30 years.

I wholeheartedly agree with Dr Day that all the animals in the world do not help us in the prevention of cancer unless they represent counterparts to man. But, by the same token, one might say that cancer in the mouse does not help us to understand cancer in the rat or dog unless these three species have something very much in common. We know from pharmacological and toxicological studies conducted with various animal species that counterparts of man's response exist in our smaller mammals. To give only one example, the rhesus monkey is excellent for the production and testing of vaccines that are used for prophylaxis of poliomyelitis in man. Pharmacologists recognize differences among species. They have learned to use certain animal organs or structures as counterparts for predicting effects for man. Thus, from carefully conducted animal studies, it is entirely possible to predict, with a fair degree of accuracy, just what a certain compound will

do to the blood pressure, to the heart, to the lungs and even to the mood of man. This type of pre-testing has been adopted so extensively that no pharmaceutical company would consider a new compound as a drug without animal studies, and no ethical industrial company would manufacture or sell a new compound without having previously obtained a report from the toxicological laboratory.

The point that I wish to make is that there are many counterparts of man in experimental animals, and that the pharmacologists have learned to make use of these in obtaining pharmacological and toxicological information applicable to man. When it comes to the solving of the human cancer problem we must admit that we have not exerted ourselves intensively enough to find suitable counterparts in the animal world.

Some scientists are unwilling to accept the concept that animal data can point to certain clues in the human cancer problem, because, at times, unjustifiable conclusions have been drawn from certain experiments such as " mouse painting tests "; but does this justify discrediting experimental animal data in general? One investigator[2] spoke of epidemiological studies and animal tests as follows: " there are a number of instances where very helpful work started with . . . epidemiologic data . . . , we then proceeded in the laboratory with animal experimental work as an adjunct or tool to help identify possible activators or methods of action in the production of cancer."

The question that I want to raise is, what makes animal experimental work a tool to help identify a carcinogen unless animal tissues had these properties *before* epidemiological studies pointed to a certain compound as a possibly carcinogenic agent? I see no justifiable reason why we should emphasize epidemiological methods instead of animal testing of a suspected human carcinogen.

Other investigators have gone to the other extreme. Dr W. C. Hueper[5] of the National Cancer Institute, would not use any chemical as a food additive which is known to have produced cancer in an experimental animal. If we accept Dr Hueper's yardstick, then we must condemn a multitude of chemical compounds, including two of our most important foods, namely glucose and fructose. When these carbohydrates are injected subcutaneously in relatively large doses into rats, they will produce sarcomas at the site of injection. Table salt will produce similar effects. It has been said that 25 per cent of all chemicals are capable of producing skin cancer in either the rat or mouse. Studies with embryonic animal tissues will show even higher incidences of carcinogenicity, somewhere between 40 and 50 per cent. Investigators who are in favour of embryonic tissue pre-cancer testing have claimed that, with this method, it is at least possible to select compounds which are not carcinogenic. But this is an unwarranted conclusion, since compounds which are harmless and, save in embryonic animal tissues, may still be carcinogenic for man.

From animal studies we have learned that:

(a) there is a direct relation between the dose of a carcinogenic agent and the appearance of cancer; this was shown with dimethyl aminoazobenzene, dimethyl aminostilbene and ionizing radiation;

(b) in contrast to the ordinary toxic effects of a chemical, the effects of a

carcinogen are apparently cumulative; therefore, " no dose "—regardless of how small—is inconsequential;

(c) local irritation, local injury, cancer-predisposing agents or so-called cocarcinogens play their rôle in the production of cancer;

(d) the length of life of individual members of an animal species largely determines the incidence of tumour formation; this is of particular significance in recognizing mildly carcinogenic agents.

For each of these four conclusions, counterparts in man could be cited. But we need additional counterparts—for instance, one or, preferably, two animal species in which we could demonstrate that a certain chemical produces the same, or a similar, malignancy as that produced in man. The bladder-cancer dog is a good example.

If I were to direct a broad cancer research programme, I would give Dr Carter's recommendation first choice, namely, to intensify biochemical studies at the cellular level. We must know what happens in cells, and these need not be human cells, before we will ever really know what went wrong in man or animal afflicted with cancer.

I would give animal studies second place, with the primary aim of searching for human counterparts. The final use of the compound and the site of its entry into the human body must dictate the details of the experimental approach. If, for instance, we are concerned with a food additive, then I would suggest that we proceed as follows:

(a) feed each compound to as many species, preferably mammals, and for as long a period of time as possible, including at least the rat and the dog and preferably other animals, but not necessarily those that have been used widely in the past; through this approach, we might discover a species more suitable than the rat or dog;

(b) start feeding the compound to weanling males and females;

(c) feed pregnant females and conduct reproduction studies;

(d) start groups large enough to provide a significant number of survivors living to a ripe old age;

(e) give careful consideration to the feeding of a well-balanced basic diet, one that will neither inhibit nor support the formation of cancer;

(f) consider administering the compound at the same time by several other routes such as application to the skin, subcutaneous injection, *etc.*;

(g) maintain a really adequate control group;

(h) add to these fundamental requirements some consideration of the strains to be used.

Evaluation of the data will not always be an easy matter, since spontaneous tumours will appear, at different sites, in treated and in untreated animals. It is for this reason that an adequate number of control animals is so critically important. If the results are significantly positive in the rat, dog, pig or monkey feeding experiment, then there is no question that the compound must be condemned as a food additive. If the results of the feeding experiment are inconclusive, then the data of cutaneous, subcutaneous or other studies may well provide the answer. If *one* mode of administration other than ingestion should induce a significant number of malignant tumours, then the data need evaluation. Whether such information should or should not eliminate a compound as a food additive will have to depend on the

species used, doses administered, method of administration or implantation, and other factors such as the importance of the contemplated use of the additive—whether it is to be added to maraschino cherries, chewing gum or dill pickles or to foods such as milk and meat.

When dealing with extremes, few people will have difficulty in reaching a decision. To quote Dr Robert Eckardt[6], " 2-acetylaminofluorene is one good example. It produces many tumours at many sites in many species and by many routes of administration. Few would argue with not permitting it in our food. But what about the material that produces few tumours in few sites in few species by few routes of administration? Here, it seems to me, the above contention becomes all-determining. If the additive is to be used in a food, like milk, which constitutes 12·5 kg out of 30 kg of processed food consumed each week by the ' average ' American family, I think we would all hesitate to use it." Living conditions and standards differ considerably in different parts of the world. For instance, an additive " might be banned in the United States where the average life expectancy is 69 or 70 years, but permitted in India where the average life expectancy is 25. While lowering the life expectancy in the United States to 60, it might raise the life expectancy in India to 40, still too low to be in the really high cancer incidence range. Also, 10 or 20 years from now it might be banned in India where once it was permitted. As our life expectancy increases to 80, 90 or 100 years, perhaps we may ban things we now accept."

In summary then, I believe that animal experiments will " do much " to give us an answer to the question of whether a certain chemical is liable to be carcinogenic when ingested by man. This is the best that we can expect since we cannot exclude the possibility that a compound, negative in animals, may still be carcinogenic in man.

Biochemical cellular studies should not be neglected since they will, no doubt, point to the mechanism of carcinogenic action. Human epidemiological studies can bring to light those carcinogens missed by animal studies; they will discover others which were never subjected to a laboratory investigation. The importance of post-mortem examinations and microscopical studies of organs and tissues showing the presence and type of cancer is equally obvious.

One final thought: *since most of us confine our research to one particular area, we are inclined to draw general conclusions from individual bits of information. Obviously, we should use all available data. In addition, it is important that we most carefully evaluate all facts and factors which surround the specific use of a material before we decide that a compound is safe or that it should not be used as a food additive.*

References

[1] W. J. Darby. Communication to the Subcommittee on Health and Science of the Committee on Interstate and Foreign Commerce of the United States House of Representatives, August, 1957

[2] E. Day. Communication to the Subcommittee on Health and Science of the Committee on Interstate and Foreign Commerce of the United States House of Representatives, August, 1957

[3] M. L. Levin
[4] H. E. Carter
[5] W. C. Hueper

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[6] R. Eckardt. Private communication