The potential toxicity of chemicals used in food technology

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Introduction

Many of the substances used as food additives possess no outstanding toxic property; others which may be used as adjuncts in processing may be toxic but are present in the food only in very small quantities. The study of non-toxic substances resolves itself into the problem of trying to prove a negative. An experimental investigation of the toxicity of many of the chemicals used in food technology may end at the point where one is left to consider the possibility that some toxic property hitherto unsuspected might eventually reveal itself among the human population exposed to the exhibition of the substance in some popular foodstuff. The question thus arises of the scientific adequacy of the laboratory methods now used to elicit the inherent toxicity of substances. The situation created by the presence in food of traces of materials known to be toxic in greater concentrations is slightly different. The problem here is whether a material endowed with specific toxic properties when given at a certain dose level can produce unsuspected chronic toxic effects if ingested over long periods in amounts not demonstrably toxic in the original way.

This paper does not attempt a survey of the toxic properties of the chemicals commonly used in food technology or to criticize current methods of toxicity testing. The first task would require a book; the second has been done on two previous occasions (Barnes & Denz, 1954; Barnes, 1955). In the hope of stimulating discussion the general problem will be briefly described and illustrated.

Simple tests for toxicity

Food manufacturers and allied commercial interests are so aware of this problem of toxicity that it is unlikely that any material with demonstrable toxic properties will ever be included in food prepared in accordance with reputable trade practices. Compounds with well-marked toxic properties and active as insecticides, fungicides, germicides or endowed with other special properties may appear in food in trace quantities as the result of current practices.

Every toxic substance has a dose-response curve, and below a certain dose no toxic effects can be detected. A safe dose will be one which the body can dispose of without harm to its normal metabolism. This safe dose must have some mathematical relationship to the toxic dose, and Gaddum (1956) has suggested a simple extrapolation from probit data. From a knowledge of the dose needed to affect one in ten animals it is possible to calculate the dose that would only affect 1 in 10,000 or 1 in 10,000,000. A 'safe dose' may be set at any arbitrary level to be determined by responsible authorities. The central problem in tests of this type is to discover criteria of toxicity amenable to measurement and less severe than the end-point of death. Loss of weight or failure to grow are liable to affect in a very uniform manner

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the small populations customarily used in toxicity tests on standard animals such as laboratory strains of rats and mice.

The difficulties are much greater when the investigator has a substance which in large doses produces no clear-cut toxic response.

Proof of harmlessness

If a substance cannot be shown to possess any toxic properties even when given in very large doses does not common sense suggest that it will also be harmless when ingested in much smaller quantities? This is not an approach that appeals to everyone. There are some who prefer to suggest that in some unspecified way the prolonged consumption of such substances over long periods may injure the health. If specific clues as to the nature of any such toxic effect were suggested, modern methods of investigation would doubtless provide means for studying them. One approach is to study the metabolism of these relatively non-toxic materials so that their ultimate dispersion, and disposal by the body may be known. Such studies may prove a major scientific investigation which in the end might only account for some fraction of the original material. The use of isotopically labelled material does not necessarily offer a ready-made solution to the experimental difficulties. An important technical problem in metabolism studies is raised by the fact that the body may dispose of small quantities of a material in a way different from that which it adopts when dealing with larger quantities. Technically it would be extremely difficult to study the metabolism of a food dye when given to animals in doses comparable to those consumed by a man eating coloured food. It would be necessary to use quantities 10 or 100 times as great. But it has been clearly shown with certain toxic substances that the disposal of small non-toxic doses may be quite different from that used when larger quantities are given (Stokinger, 1953).

Sprout depressants on potatoes

Despite these reservations some knowledge of the metabolism and mode of action of a chemical substance offers the most promising approach to an understanding of any toxic properties it may possess. Potatoes are a staple food, but in bad seasons or during emergencies stocks in this country do not always last for a full 12 months. This situation could be much improved if the sprouting of stored potatoes were prevented and their palatability and quality thereby preserved, which can be done by dusting the potatoes with certain chemicals. One such substance is maleic hydrazide. It has a most striking effect on many plants, inhibiting their growth without killing them. Despite a widespread effect on plant growth at concentrations of a few p.p.m., rats and other animals will grow and thrive on a diet containing as much as 1% maleic hydrazide. When used properly, residues of 10–15 p.p.m. may remain in the potatoes at the time they are consumed. Maleic hydrazide is not destroyed during cooking. Is it safe to permit or encourage its use as a sprout depressant on stored potatoes? It has been impossible to demonstrate any toxic action on animals, and in the rabbit at least 40% of a large dose is excreted unchanged

(Williams, 1955). Clearly it would be helpful if something was known about the action of maleic hydrazide on the plant. On seedlings studied in vitro it acts as an anti-auxin (Leopold & Klein, 1951). The auxins, such as indoleacetic acid, allow the plant cells to grow and divide normally, and indoleacetic acid will counteract the effect of maleic hydrazide on seedlings. It has been suggested that maleic hydrazide exerts its effects on plants by stimulating the enzymic hydrolysis of indoleacetic acid, so depriving the plant of its auxin (Waggoner & Dimond, 1953). If this suggestion can be substantiated the specific effect on plants is explained, thus suggesting that the negative results of toxicity tests on animals truly reflect the non-toxicity of this substance to mammals and man. However, the study of maleic hydrazide raised another problem all too commonly met in this field. The cytological changes in plant cells affected by it included chromosome splitting. As many carcinogenic substances have a similar effect on mammalian cells, it was suggested that maleic hydrazide should be tested for its carcinogenic effects before being used on food for human consumption (Darlington & McLeish, 1951). In a preliminary test on a small number of rats three sarcomata distant from the site of injection were produced. This element of doubt led to the need for a more comprehensive test (Haddow, personal communication). In addition to a long-term test on rats and mice by feeding and by injection, other tests on the effect of maleic hydrazide on mammalian cells in vitro have been done (Bullough, 1955; Cruickshank, 1955). The former test is not quite complete; the latter tests showed it to be without effect on dividing mammalian cells. The uniformly negative result of tests on mammals suggests that on potatoes in a concentration of 10–15 p.p.m. it would not be harmful though it does not amount to proof that it would be entirely safe. Tetrachloronitrobenzene has also been recommended as a sprout depressant. A number of tests on the toxicity of this substance have been carried out (Buttle & Dyer, 1950), and its metabolism has been studied in some detail (Bray, Hybs, James & Thorpe, 1953). However, it is steam-volatile, and further discussion on the possibility of the small residues being toxic was rendered unnecessary by the knowledge that any residues remaining on the raw potato would be volatilized and driven off when the potato is cooked. It is clearly important to understand the physical and chemical properties of substances added to food, as these may play just as important a part as the biological properties in determining whether a health hazard will be associated with their use.

Surface-active agents

Anxiety about possible carcinogenic effects dominates much of the thinking in this field of chronic toxicity of chemicals. There are comparatively simple acute tests such as the effect on body growth, tumour growth and cell division which seem adequate for distinguishing powerful carcinogens. A definition of a 'weak carcinogen' has not yet been provided. Since cholesterol has been shown to have carcinogenic properties (Hieger, 1947) it might serve as a kind of standard. A diet of milk and eggs will produce liver tumours in the rat (Nelson, Szanto, Willheim & Ivy, 1954) but in man it might be more likely to produce atherosclerosis. In America there has been some controversy about the alleged hazards of adding certain emulsifying agents

to bread in order to keep it soft. The type of toxicity tests upon which some of these arguments are based has been criticized before (Barnes, 1951). These surface-active agents may have interesting biological effects, but whether or not these effects will be shown by the emulsifiers when present in bread has not yet been determined. When added to the diet of rabbits receiving cholesterol the emulsifiers increase the amount of cholesterol absorbed and raise the blood lipid levels and increase atheroma (Kellner, Correll & Ladd, 1948). When emulsifiers were injected the deposition of lipid as atheromatous deposits was decreased. Injected into rabbits on a normal diet, however, they are said to raise the blood lipid and increase atheroma (Kellner *et al.* 1951*a*). Thus their role in lipid metabolism is by no means clear (Kellner *et al.* 1951*b*). An appraisal of the potential toxicity of this group of substances should include some study of their effects on the absorption, transport and deposition of fat.

The toxicity of agenized flour

Not only may the chemicals added to food become altered but so too may the food itself, and the products of such reactions might be toxic. The classical example is the agene process for 'improving' flour. Can the discrepancy between the experimental observations on the dog and the apparent harmlessness of agenized flour for man be explained? Agenized flour was shown to be toxic to dogs, cats, rabbits and ferrets. Rats and monkeys were relatively insensitive. Observations on nineteen human beings including five epileptics who were given flour treated by twenty to thirty times the usual quantity of agene showed no change in behaviour, general physiology or the electroencephalogram (Newell, Erickson, Gilson, Gershoff & Elvehjem, 1949). When the active toxic agent, methionine sulphoximine, was isolated the sensitivity of animals was further checked. The dose required to induce fits in the monkey is at least 100 times as great as that needed in the dog. A man eating 2000 Cal. daily as agene-treated flour ingests a dose of 1 mg methionine sulphoximine/kg (Gershoff & Elvehjem, 1951*a*). The sensitive dog requires a dose of 2–4 mg methionine sulphoximine/kg to produce fits and kill the animal, but the dose-response curve must be steep, for on a diet containing up to 30% of agenized flour dogs lived in perfect health for a year and showed no electroencephalogram changes (Newell *et al.* 1949). Unfortunately it is not yet possible to understand exactly why the dog and man differ in their sensitivity to methionine sulphoximine. It is known that methionine in much bigger doses can counteract the effects of methionine sulphoximine (Reiner, Misani & Weiss, 1950) but the nature of the toxic action of methionine sulphoximine has not been defined. Studies in vivo and in vitro have failed to demonstrate a specific effect of methionine sulphoximine on brain metabolism (Gershoff & Elvehjem, 1951*b*) but it does interfere with glutamine metabolism probably by interfering with the glutamyl-transferase system (Pace & McDermott, 1952). It is known that glutamine metabolism is very important in the brain but it is probably equally important in the brain of all species. It seems therefore most likely that the species differ not in the sensitivity of their metabolic process to methionine sulphoximine but in the speed and ability with which they dispose

of the toxic substance. It is unfortunate that in recent studies with labelled methionine sulphoximine the rat has been used (Roth, Wase & Reiner, 1952; Roth, Wase & Eichel, 1953). The fact that it did not accumulate in the brain may be a reflection of its rapid destruction in this resistant species. Even in the sensitive dog, death may not occur for from 2–4 days after a single dose which suggests that methionine sulphoximine may need to persist to show its toxic effects. If differences in the distribution and rate of metabolism of methionine sulphoximine in the different species do exist, they might explain why man is not poisoned by a prolonged consumption of agenized flour.

Insecticides on stored grain and crops

The search for toxic metabolites in flour treated by chlorine dioxide has been unsuccessful so far (Frazer, Meredith & Sammons, 1953) and there are no positive tests from feeding animals that would help in making this particular search. The action of agene, however, stimulated work in another field. Methyl bromide has been used as a fumigant for stored wheat. As it was known that bromine was liberated when methyl bromide came into contact with wheat grain, Winteringham and his colleagues embarked on a search for products of this reaction (Winteringham, Harrison, Bridges & Bridges, 1955). In a series of experiments which have been described in detail they found that 80% of the methyl bromide that reacted did so with the wheat gluten. Of the products so formed 50% were N-methylated compounds, 30% dimethylsulphonium derivatives and the remainder methoxy and thiomethoxy compounds. Further work showed that it was histidine that was methylated either as the 1-N methyl, 3-N methyl or 1:3-N dimethyl derivative (Bridges, 1955). These methylated products were then prepared and isolated and tested for their toxicity. The final paper of the series gives a detailed analysis of the possible hazard that might arise from the use of methyl bromide as a fumigant. Although less than 1% of the wheat used in this country is treated with it it was assumed that a man might consume a diet containing 1·75 kg of treated flour per week. It would contain 175 mg of the decomposed methyl bromide in different forms, the major ingredient being 147 mg of bromide. Details of all the other reaction products are provided and from existing knowledge it was possible to provide evidence of their probable harmlessness (Winteringham, 1955). This work is a model of what is needed in order to assess a possible hazard from a new technological process for food where chemical reactions between the food itself and chemicals introduced from outside might be suspected to produce toxic metabolites. However, had the methylhistidines discovered during this work been shown to be toxic then a whole new series of experiments would have become necessary before the hazard could have been properly assessed.

Anxiety is sometimes expressed about the use of the so-called systemic insecticides which are taken up by the growing plant and, while they remain in the juices of the living plant, kill insects feeding on them. Chemists and biochemists have tracked down these substances after they have entered the plant. For one known product—demeton (*O*,*O*-diethyl-*S*-ethylthioethanolthiophosphate) —it has been shown

that the metabolic processes are exactly the same in the plant, the insect and the mammal (Metcalf, March, Fukuto & Maxon, 1955). With schradan (octamethylpyrophosphoramide) very little metabolic change takes place in the plant and the toxic metabolic products of schradan do not accumulate because they are unstable (Heath, Lane & Park, 1955). However, in both the mammal and the insect metabolism, which follows the same pathways, is so much more rapid that toxic effects are observed.

Conclusions

In all applied toxicological work of this type it is difficult to overemphasize the importance of the chemist and biochemist in devising methods suitable for the detection and estimation of biologically active contaminants in such a chemically complex environment as food. Toxicity is not a specific measurable characteristic like the melting point or molecular weight of a substance. The toxicity of a substance is related to the other conditions in a living system which is exposed to its influence. Clearly it is essential to have an accurate estimate of the quantities of any such materials that might be consumed, and if toxic effects can be demonstrated in animals, generous safety factors must be allowed before a safe dose for man can be suggested. Where no positive effects can be observed on animals then a hypothetical risk may be incurred if such a chemical is added to food. This hypothetical risk should in some way be balanced by the simultaneous conferment of some benefit to those who incur it.

The duty of the toxicologist must be to guide those who wish to introduce a new technical process into food production or manipulation so that they will avoid exposing the consumer to any foreseeable risk of intoxication however mild in degree. It would seem to be the duty of those with a training and experience in nutrition to advise whether or not a proposed new process has merits or dangers on other grounds. Their opinion in turn may be modified by what they can learn about the possible toxic hazards involved.

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Unexploited technological possibilities of making food for man and animals

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The three outstanding possibilities are: the synthesis of food, the more economical use of crops of the type we already grow, the exploitation of new primary sources of food. There is no need to discuss the first here. Experiments on fat synthesis are described by Williams (1953), and Cuthbertson (1953) has stressed the importance of synthetic vitamins and amino-acids in turning a nearly adequate diet into an adequate one. Both agree that synthesis of the bulk foods is not likely to become an important feature of food production soon. We will continue to rely mainly on photosynthesis.

When we eat a plant the digestible part is, by definition, used with 100% efficiency. Few people get less than half their energy from vegetable sources and most of the world's population gets nearly all its energy in this way. One solution to the food problem would therefore be to increase the amount grown. Even if this were done there would still be room for improvement because much of the crop, e.g. leaves, straw and peel, is wasted. Furthermore, the diets eaten in many parts of the world are inadequate. They have many faults but protein deficiency is a common one and the steps that could be taken to overcome it will serve as an example of what could be done to remedy the other deficiencies also.

The parts of a plant generally eaten are the starch depots such as grains and tubers. Some legume seeds contain adequate amounts of protein but many tubers contain very little; cassava with only 1–2% is an extreme example. As would be expected from their active metabolism, immature flowers and young leaves can be rich in protein, but they are inadequately exploited. In Britain brussels sprouts and cauliflower are the most valuable materials of this type eaten in significant quantity. This conservatism may now be unnecessary because refrigeration has solved the old problem of storing materials as perishable as leaves. In Table 1 a few