Testing New Drugs in the Laboratory

D. G. DAVEY, OBE, MSc, PhD

Formerly Research Director, Imperial Chemical Industries Ltd, Pharmaceuticals Division

The importance of drugs needs emphasising because they receive a great deal of criticism nowadays, particularly from people who do not need them. If a disease is not preventable we must try to find a treatment for it. Some drugs are little short of marvellous; others leave a great deal to be desired. Mental illness, skin disorders of various kinds, rheumatoid arthritis, and so on, cry out for better treatments, and for some diseases, notably the sequelae of diabetes and multiple sclerosis, we can do little or nothing. The search for new drugs must therefore continue.

We cannot blindly test new chemical compounds in man, hoping one may be a drug. The preliminary search, the sorting out and pursuit of ideas must be done in laboratory animals. This needs a model of the disease in a convenient laboratory animal. The model in the laboratory animal is a reflection of current knowledge of the disease as it occurs in man. For some diseases the attempt to establish a model, or a testing system embodying a critical factor in the development of the disease, is a most salutary exercise. What lies behind schizophrenia? What lies behind depression? What is the sequence of events leading to rheumatoid arthritis? What are the causes of hypertension in man and which are the most important? The questions are posed to emphasise our ignorance, but fortunately our ignorance is not complete. To all the questions we could give some sort of answer, sometimes only partially right, possibly sometimes wholly wrong, but ideas are there and they must be pursued if we are to advance.

It is because there is still so much to learn about living matter that the discovery of a good drug is so difficult and the research seemingly so wasteful. Ten thousand compounds may be synthesised, each one with hope, before a new drug is discovered. The precise order of magnitude does not really matter but the number is so big that some people advocate stopping the search for more drugs until we can make the search more rational and more certain. I do not agree with this view. Even a small advance may benefit a large number of patients, but it will also benefit research by pointing the way forward to further advance. This playback from the clinic to the laboratory has been all-important in the discovery of drugs and it will be a sad event if the desire for unattainable perfection should interfere with the disciplined exploration that characterises good experimental medicine.
When what appears to be a drug — it is properly called a candidate drug at this stage — has been discovered in the laboratory it must be tested for safety; that is, the risks of untoward or adverse effects if it is given to man must be defined. It is a mistake to suppose that safety testing was not done until after the thalidomide disaster, at least by the reputable pharmaceutical companies. What thalidomide did was to cause most countries to establish a regulatory authority whose duty was to see that safety testing was done on all new drugs to the best standards prevailing. It is also a mistake to suppose that safety testing is done to exclude any drug that exerts toxic effects. All drugs are toxic in some circumstances, and the purpose of the safety testing is to define the circumstances in which toxic effects are manifest and then to judge whether such circumstances will be encountered in use. This precept was not followed in the case of thalidomide, which was the chief reason for the disaster. The drug was promoted for use in pregnant women and it had not been tested in pregnant animals to determine any effect on the fetus.

Another example of trying to foresee what might happen with a drug in use is the story of the mishaps, sometimes very serious, which occurred with the monoamine oxidase inhibitors, introduced for the treatment of depression. As their name indicates, they inhibit an enzyme that breaks down monoamines such as noradrenaline and adrenaline. Tyramine is a monoamine prevalent in certain foodstuffs, particularly cheese where the content is relatively high. Normally, tyramine is broken down in the alimentary canal and in the wall of the canal, and little reaches the circulation. The monoamine oxidase inhibitors prevented this breakdown, relatively high concentrations of tyramine were then absorbed into the systemic circulation, and dire consequences followed. The presence of tyramine in cheese and other foods was known long before the monoamine oxidase inhibitors were launched as drugs, but clearly it would have needed a very knowledgeable, indeed a very prescient person, to have foreseen what would happen.

The magnitude of a dose that produces a toxic effect obviously influences judgement. How near is this dose to the one that it is thought will be used therapeutically? In other words, how much latitude is there to cater for any patient who might be more sensitive or for the mistakes that will inevitably occur in usage? Far too many people, including doctors, believe that if a particular dose does good, twice or three times the dose will do much better. There can be no fixed rule on latitude because it depends on how a drug is used; for example, most, if not all, anaesthetics have a relatively small latitude, and a concentration four or five times the anaesthetic concentration might kill. But anaesthetics are in the hands of skilled people and it is unthinkable that they would err so much.

Finally, in exercising judgement, one must take into account the condition to be treated. Is it serious or trivial? Obviously, risks can be taken in the treatment of cancer that cannot possibly be taken in the treatment of threadworm in children. What other treatment is available? Take as examples the anti-inflammatory drugs.
used for rheumatoid arthritis, or the beta-blocking drugs used for arrhythmias, angina and hypertension. There are ten or so anti-inflammatory drugs, all more or less in the same mould, with minor differences in effectiveness and disposition to cause adverse effects, although sometimes the minor differences are important to some patients. There are also about as many beta-blocking drugs, most of which are again in the same mould. If a new anti-inflammatory or beta-blocking drug is discovered that belongs, to all appearances, to existing moulds and there is the possibility that its use would present some risk, proceeding to the clinic is not justified.

Testing a drug for safety is, when done properly, a study in its own right and it might not follow precisely a study of another drug done previously or a study of another to be done later. In each case these questions must be asked: what disease or diseases will the drug be used for, how will it be used, what particular circumstances might it encounter, and will the envisaged study be likely to satisfy the points raised by these questions? Of course, each study embraces a similar basic pattern, one that has evolved, rather arbitrarily, over the last 40 years or so, during which the study of safety testing of drugs has developed. But parts of the pattern deserve special attention for some candidate drugs, less attention for others.

First, the LD50 (i.e. the dose killing approximately half the number of animals receiving it) is measured in several laboratory species. My opinion is that, except in very exceptional circumstances, a very accurate measurement, say within 95 per cent confidence limits, is not called for. It would be necessary if the LD50 were used for biological standardisation, a relatively rare occurrence nowadays, or if someone wished to commit suicide as scientifically as possible, but an approximate LD50 is all that is usually required. It can yield valuable information; in particular, it shows if the different species behave similarly towards the drug or if there are marked differences. If there are differences the causes must be sought.

Next, a specific method for measuring the concentration of the drug in body fluids and tissues must be found. With modern instrumentation and technology this is usually not too difficult, and it is essential to have the method if prolonged testing of the drug in the chosen species is to be done intelligently. Incidentally, modern instrumentation, particularly the mass spectrometer and the nuclear magnetic resonance machine, has led to drugs being among the purest entities ingested by man. These instruments detect very small amounts of impurities which can then be removed. Looking at an analysis of a cabbage, I reflected that if it were a new entity and submitted with its analysis to a regulatory authority it would never get acceptance.

Few drugs nowadays are given on a single occasion and many are taken for very protracted periods, often for the remainder of the patient's life. Prolonged tests for toxicity are therefore essential. There is argument about how long the period should be for drugs that will be taken by a patient for many months or years.
Excluding special tests for carcinogenic potential, some workers think three months is long enough to detect conventional damage to organs; others say six months and a few say one year. The choice of period is arbitrary and unproven.

Rats and dogs are most often chosen for these prolonged experiments and the reasons are almost purely historical. At the time when toxicity testing was in its infancy rats were generally healthier than the mice available and mice or rats were desirable because they are so easy to house and handle. In those days, analytical methods were not nearly as sensitive as they are now, so the larger blood volume of the rat was also an advantage. A second species was introduced because species vary in the way they react to drugs, and someone decided that the second species should be a higher species. The advantage of a higher species lies in the higher development of the central nervous system and, outwardly at least, it is easier to discern an adverse effect on the central nervous system or sensory organs of the dog than of the rat. Dogs were chosen because in those days they were cheap; they were unwanted mongrels from the street. Nowadays the dogs are almost entirely specially bred for laboratory work and are mostly beagles. If toxicity testing had originated in India the second species would have been the rhesus monkey.

Variation between species is a basic problem confusing the collection of data in laboratory animals and the attempted transfer of the data to man. Many millions of years ago when vertebrates left their aquatic environment and took to the land they were perforce vegetarian and had to learn how to deal with the extraordinary variety of chemicals they encountered in the plant world. They were extremely successful in accomplishing this and the enzymatic processes they developed alter in one way or another, to a greater or lesser degree, almost every chemical compound there is, whether designed by God or man. This is the metabolism of a compound and very few compounds indeed, assuming they are absorbed, pass completely unchanged through the body. Unfortunately, details vary between species. Different amounts of the compound may be metabolised and different metabolites formed. With drugs, it is usual for the parent compound to be most toxic, and the metabolites, commonly water soluble, are, with a few exceptions, quickly and harmlessly excreted.

During the last 30 years a great deal has been learnt about the metabolism of compounds and now it is possible to obtain an overall picture of what is happening to a candidate drug in laboratory species without insuperable difficulties. Much time, money, and technical expertise may need to be expended, but the work can be done. A specific method for measuring the drug must be developed. It is also necessary to synthesise the drug to carry a radioactive atom as a radiolabel. The label is placed in such a position in the molecule that all metabolites probably carry it; two labels may sometimes be necessary. The labelled drug is then given to the animal and measurements are made of the drug itself and of total radioactivity in the blood. If the two sets of measurements
approximate, little metabolism has taken place; if they diverge greatly, much metabolism has occurred. Measurements are also made over several days of radioactivity in urine and faeces and this should be done in animals with a cannulated bile duct so that one can say if drug in the faeces is unabsorbed drug, or drug excreted in the bile. Chromatography is done on the urine and the metabolites and their approximate relative concentrations.

One more complication must be mentioned. Some compounds stimulate their own metabolism so that after particular doses given repeatedly the later drug concentrations are less than they were at the beginning. Other compounds may be slowly excreted and too frequent dosing may lead to their accumulation. Measurements must therefore be done at different times during the course of the tests and not simply at the beginning.

The metabolic investigations must be done in rats and dogs and, later, in man as this is the only way of knowing how much of the work done in the laboratory species is relevant to man. I find this fact very worrying and believe the time has come to review what has been learnt about toxicity testing and to question the whole procedure.

Rats and dogs will usually be represented in the prolonged tests by three medicated groups with a control group. The lowest dose of drug administered will be somewhat above the estimated therapeutic dose or the dose giving the estimated therapeutic blood level; the highest dose will be the biggest that can be given without causing or threatening to cause death, and there will be an intermediate dose. At intervals during the treatment of the animals standard clinical chemical tests and chemical pathological tests will be done, and at the end of the period of treatment the animals will be sacrificed and samples of all tissues taken for histological examination.

Special tests, which include carcinogenic tests, mutagenic tests, teratogenic tests and reproduction tests, are also done. Carcinogenic tests are done usually in rats, mice and sometimes hamsters, because it is impractical to use dogs and monkeys. There are mysteries about carcinogenesis — the long latent period before tumours develop, the production of tumours seemingly only after often repeated dosing — so, until we know more, we will have to treat a species for most of its lifespan to be sure of the results, which means 18 months for a mouse, 2 years for a rat and 12 or 15 years for a dog or a monkey. At present the way we do some of the carcinogenic tests and the interpretation we give to the positive results, are, in my opinion, unreal and have little relevance to what would happen in man.

A cancer cell is really a mutated normal cell, so carcinogenesis and mutagenesis are related subjects. However, a mutagen in the exact sense of the word, i.e. a compound affecting only the gonadal cells, must be a relatively rare event. An awareness of mutagenesis underlies part of the design of reproduction tests done,
again for practical reasons, in rats and mice. *In vitro* tests for mutagenicity, using bacterial and mammalian cell systems, are the subject of much research and may lead to more rapid tests for carcinogenicity.

Finally, although not necessarily last in the sequence of events, there are teratogenic tests, usually in rats and rabbits or marmosets. Whenever a different species is introduced into the scheme of testing, further pharmacodynamic and metabolic work should be done and, in the teratogenic tests, it should also be known if the drug actually crosses the placenta and is present in the fetus.

This survey of the laboratory testing of a new candidate drug has indicated the problems and shown that there is a great deal of work done, much of it very demanding of skill and intelligence. The value of this work in protecting the public from danger is apparent from the fact that far more candidate drugs are rejected in the laboratories where they are investigated than are rejected by regulatory authorities. Whether rejection was the correct course to take in every case will never be known but the chances are that the rejections made by pharmaceutical research workers have saved patients from adverse effects. But adverse effects are still prevalent, sometimes with tragic consequences, with the use of drugs that have passed the originating laboratory’s standards and those of the regulatory authorities.

Often the so-called side effects are part and parcel of the particular therapeutic approach, and it is the inadequacy of the approach rather than the inadequacy of toxicity testing as such that leads to them, and they will be removed only by improving the therapeutic approach. The history of the treatment of hypertension provides a good example of this. Several years ago the ganglion blockers were introduced for this condition. They blocked a major part of the autonomic system. They certainly lowered blood pressure but inevitably they did much else. The next stage was to halve the side effects, as it were, by introducing drugs that affected only the sympathetic system, leaving the parasympathetic unaffected, but they still affected more of the sympathetic system than was necessary to control hypertension. Hopefully, this situation has still further improved. Another example is provided by the existing anti-inflammatory drugs used in the treatment of rheumatoid arthritis. All of them cause trouble in the alimentary canal, sometimes ulceration. To avoid this, drugs must be found to alleviate rheumatoid arthritis by a different mechanism. To succeed we must know more about rheumatoid arthritis, not about toxicity testing.

In these cases, the bad effects were foreseen and the choice of using or not using the drug was given to the doctor — did the benefit conferred by the drug outweigh the potential risk arising from its use? Let me end with an example of a drug producing serious side effects that were not foreseen. I refer to practolol, which was a deep disappointment to me. The aim of drug research is to discover compounds that are more and more specific in their effects, compounds that accomplish only what is necessary to control a disease or a symptom. Only in this
way is there a chance of overcoming the problem of adverse effects. I regarded practolol as an approximation to the perfect drug. It had little effect on the peripheral circulation, little effect on the bronchial tree; most of its beta-blocking action was confined to the heart. It also had quite extraordinary patient acceptability. Then came the bolt from the blue and we learnt that it could produce in a small proportion of patients a most bizarre syndrome, which could embrace the skin, eyes, inner ear, and the peritoneal cavity. There is still no explanation for this, although one supposes that it must have an immunological basis. With present knowledge we cannot say that it will not happen again with another drug.

Any drug that, like practolol, produces some serious idiosyncratic effect in a very small proportion of patients, presents a special problem for laboratory testing. Assuming that there was a species of laboratory animal suitable for the detection of a suspected idiosyncratic reaction, there would be the problem of coping with experiments on 50,000 to 100,000 animals.

DISCUSSION
Dr A. Herxheimer did not think it enough to say that a new drug in the mould of others should not be tried in man if there was any special risk attached to it. A new drug ought to offer something different, a positive advantage, before it was used in man. Dr Davey said it was not as simple as that because a new variation might be more acceptable to patients and it was difficult to bar a drug unless there was a specific reason. For example, practolol did not enter the nervous system but propranolol did. If cardio-selectivity was the criterion, propranolol might be banned, as it reached the nervous system. Such a ban would have prevented the promising trials of propranolol in the treatment of schizophrenia.

Mr M. S. Thomas, M.P., was concerned with the methods by which general practitioners were informed of experimental work on new drugs. There had to be a better system of explaining the pros and cons of a drug. Advertisements were not written by the research department. There was a natural tendency to accentuate the benefits and a reluctance to accentuate the negative side of a new drug. This did not provide an efficient mechanism to aid the GP in his decisions.