The Principles of Humane Experimental Technique

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CHAPTER 5

REPLACEMENT

With respect to the "analogical" ... resemblances between organic beings ...

The Uses of Tissue Culture

Tissue Culture in Virology

The discussion so far has been rather academic. Without attempting a comprehensive survey we may make this chapter a little more concrete by considering two of the major replacing techniques: the use of tissue cultures (with other *in vitro* tissue and organ techniques), and the use of microorganisms. We shall begin with tissue culture, and first with its most successful application to date--the use of tissue culture in virology. (For much of the content of this sub-section we are indebted to personal communications from F. K. Sanders.)

The use of vertebrate hosts in virology must often involve direct inhumanity. In the past few years, however, a more publicized hazard has been the *contingent* inhumanity involved in the transport of quantities of monkeys from the Far East. Of these only a very few were used for *in vivo* testing, the vast majority being required to furnish kidney tissue for culturing. The contingent conditions of transport have been disturbing and inhumane (Stevens, 1955). Steps were taken to improve matters: the Indian Government imposed salutary regulations (Stevens, ibid.), and M.R.C. issued recommendations on humane shipment (summarized: Anon., *UFAW Courier*, 1955) which were adopted by all the British airlines concerned with livestock transport[1]. It is pleasant to notice such action being taken on behalf of animals which, although our near relatives, receive none of the privileges accorded by the Home Office to cats, dogs, and the equidae (the commonest animals to be encountered in urban Victorian England)--despite the associate of both Darwin and Huxley with the movement of 1876. The monkey transport problem is, however, far from solved. It is therefore encouraging to note that rapid advances in technique (e.g. use of human tissues) may eventually bypass the problem. Meanwhile it has been

shown that monkey kidneys can be transported by air in the form of trypsinized cell suspensions which remain bacteriologically sterile and can be cultured on arrival (Sanders and Hoskins, 1955; Melnick *et al*, 1956; Sanders, 1957). This may, in time, eliminate the need for transport of whole live monkeys.

The actual use of live animals in virology is never protracted longer than strictly necessary, and the progress of replacement of this field has been unparalleled in its vigor. Virology is peculiar in that its advance has almost entirely hinged on the provision of techniques which are devoid of direct inhumanity. Viruses will only grow in living cells, but their study in vertebrate hosts is beset with a forbidding array of difficulties and complications.

"In most cases the source of virus has to be crude suspensions of infected tissue which contain much apart from active virus particles. The tissue inoculated contain many kinds of cell differently situated as regards susceptibility and availability for infection; the number of susceptible cells reached by a given inoculum is almost unknown. And, finally, the whole result may be complicated by nutritional and genetic factors, or by immune response on the part of the host organism" (Sanders, 1952).

In such a field, a start can barely be made without the provision of models of high discrimination, which permit control of many of the variables concerned at the cost of eliminating others (cf. Dulbecco, 1955). And (see below) culture methods afford other great advantages. Virus workers have, therefore, been under a powerful incentive to develop means for studying viruses outside the living animal, and have made great strides towards this end. There are some viruses--e.g. measles, chicken pox, the APC (adenoidal, pharyngeal, conjunctival) group, and polio itself--which *cannot* be studied *in vivo*.

The growth of viruses and Rickettsiae on the chick embryo was seriously started in the thirties, and by 1952 Cox was able to list a great number of them which can multiply and damage one or other part of this system, as well as a number of vaccines produced in this way. More recently, de-embryonated eggs have been used (Barnkopf, 1949). There are, however, serious objections to the chick egg or its embryo as a general tool. Many viruses will not grow on it, others will grow but without causing easily detectable damage; more seriously and generally, it is unsuitable for quantitative work (cf. Dulbecco, 1955). The same is true of the earlier types of tissue culture, in which more than one type of cell is present and surface conditions are complex.

"The last few years have been a turningpoint in the field of animal virology" (Delbucco, ibid.). The use of cell suspensions in roller-tubes (e.g. Sanders, 1953) and monolayers of cells on glass (e.g. Plowright and Ferris, 1957) are making for rapid

advance. "With the present emergence of tissue-culture technique as an almost standardized procedure and the availability of animal virus suspensions of high titre, it has become feasible to undertake quantitative metabolic studies with animal viruses, and to relate any observed effects with virus growth" (Levy and Baron, 1956). The actual turning point might well be marked by the discovery of Enders, Weller, and Robbins (1949) that polio virus, hitherto the despair of virologists, could be cultivated in cells grown *in vitro.* This discovery, for which its authors were awarded the Nobel Prize, not only made possible at once a whole series of developments in polio research and its applications, culminating in production of the vaccine, but stimulated a whole crop of researches of the same general kind. The result has been a steady recruitment of methods for culturing other viruses (Enders, 1954; cf. Sanders, 1954).

Sanders (1957) has raised the interesting historical question, "Why should tissue cultures have come to be used on a large scale just at this particular time?" As he shows, it was known in 1913 that viruses would survive in isolated tissue, in 1925 that they would multiply there, in 1931 that virus prepared in such culture was effective for human vaccination, and in 1943 that cytopathic changes (that is, visible changes of cells due specifically to a virus) could be seen in cultures as indicators of the infectivity of virus preparations. "Yet none of these discoveries was followed immediately by the adoption of tissue culture techniques in virus laboratories all over the world, or caught the imagination in the same way as Enders and his collaborators' work only a few years later." One reason, he suggests, lies in the fact that in those early days:

"tissue culture had been the province of the artist in biological technique. This was because the strict precautions which had to be observed to exclude contaminant microorganisms from the cultures discouraged all but the most fastidious, careful, and persistent workers ... and limited the number of manipulations that could be performed on individual cultures."

All this had been completely changed at the time of the polio discovery by the advent of *antibiotics,* several of which were found to be without effect on most viruses. Tissue cultures could, therefore, be maintained, and viruses grown on them, without any of the elaborate equipment and operations previously needed, simply by adding sufficient concentrations of antibiotics to suppress the growth of bacteria. Thanks to these agents, "given an incubator, a balance, and an adequate supply of domestic utensils, even an amateur in his kitchen can do it". Experimental biology and experimental animals have thus benefited in an unexpected way from the exploitation of antibiotics stimulated by medical demands in the World War II. The limitation removed was, as we might have supposed, a purely technical one, of that interesting kind that prevents *large-scale* application of an intrinsically possible method. The repeated occurrence of a type of discovery which had no great effect the first time and

profound influence on the second occasion, thanks to the appearance meanwhile of an apparently unconnected technical improvement, is not rarity in the history of science.

At present there are only two purposes for which live higher animals must be used in virus work. Virulence is a complex property of virus-host systems, even less understood than that of bacteria, and high fidelity is still needed here. In culture polio grows in kidney tissue, while *in vivo* it selectively invades the nervous system. Strains of virus with full antigenic properties may fail to invade the normally affected tissues. Live vertebrate hosts are therefore still used to test the virulence of virus at certain stages of vaccine production and in some research problems.

Second, animals are used for the production of antiviral sera. The viruses used are killed, or produce symptomless infection. This is not in itself, therefore, a case of direct severity. At the same time, it is interesting to note a recent discovery--the possibility of eliciting antibody production in a totally *in vitro* system composed of spleen tissues (Stevens and McKenna, 1957). This mode of replacement might have considerable importance for contingent inhumanity in the general context of antiserum production.

Even in virulence tests, culture methods have ensured a great reduction in the number of animals used. There is now no need to titrate virus quantitatively on animals--a procedure involving large numbers of the latter--since quantities of virus can be accurately *counted* by culture methods. Thus known dosages of virus can be inoculated in test animals. For many viruses, *all quantitative* work is now done on tissue culture.

For all other purposes (growth, identification, serological study, vaccine production, etc.) use of cultures is always better and often obligatory. Syverton and Scherer (1954), working with a strain of human cells, list nine broad purposes for which these cells can be used "readily, inexpensively, and effectively".

The spread of culture methods in virology is now limited by only two factors. The first is the need to discover a suitable tissue for growing a particular virus. The second is the logistic problem where large-scale, e.g. vaccine, work is concerned. Large-scale tissue culturing even in the kitchens' age still requires some initial preparation of a laboratory, and there is a shortage in this country of trained workers. Vaccinia vaccine in Britain is entirely produced from calf lymph. The virus has been grown in tissue culture for some time (cf. Crawford and Sanders, 1952), and in Sweden vaccinia vaccine has been produced from tissue cultures of bovine embryos obtained from pregnant cadavers in slaughterhouses. Fortunately, it seems likely that progress in the extension of culture methods is beginning to accelerate.

Among viruses which still cannot be grown *in vitro* are trachoma, dengue fever, and smallpox. Among important viruses now regularly grown *in vitro* are polio, measles, chickenpox, the APC group, and the important veterinary diseases Newcastle, fowl plague, foot and mouth, vesicular stomatitis, and dog hepatitis. Of special interest are the ECHO viruses (Enteric Cytopathic of Human Origin), also known as "orphan". These have turned up from human pathological material inoculated into tissue cultures in a search for polio virus. They were not at first known to be correlated with particular human illnesses; at least one such connection has now been traced. Their discovery would have been impossible without tissue culture methods. Another group of which this is also true is that called adenoviruses, now known to be connected with certain cases of respiratory disease in man (cf. Sanders, 1957).

The progress of replacement in virology has recently been reviewed by Sanders from the humane point of view (1957). He discusses in particular the quantitative aspect. The crudest method of counting the number of virus particles in a sample employs living animals deliberately infected, different groups being dosed with different dilutions of the sample, and the relative number of deaths or symptoms in each group used to estimate the number of particles in the undiluted sample. Each animal, by showing or not showing symptoms, provides exactly one bit of information in the technical sense.[2] But one animal can provide say, 100 tube tissue cultures. If the same method is used on these, instead of on whole animals, each culture provides one bit (the cells are affected or not), but each animal provides 100 bits. In the acquisition of this information, no trouble now arises from differences between animals (in e.g., susceptibility). Such methods have also resulted in the discovery of the new viruses mentioned above, and the fluids of such cultures often contain virus in high concentration together with very little host protein, so that they are a better source of virus for future inoculates.

Both these methods are, however, estimates, and involve a tortuous way (via theoretical continuous variables) of arriving at a count of what are in fact discrete particles. Neither can ever be completely precise. It is, therefore, of great interest that two new techniques have been evolved by which the virus particles can be counted directly. Now bacteria has long been counted simply by being spread out on a plate. By taking advantage of this, it has similarly been possible to count bacteriophage particles. Where animal viruses are used, animal cells must, of course, be employed as indicators, and the technical difficulty arose here of making them spread out and stick where they fall. This difficulty has now been overcome in two different ways, which enable a *single* virus particle to make itself felt as a differentially stainable spot in a sheet of animal cells, so that the particles can simply be counted as spots. A single plate of this kind can provide as much information as 500 tube cultures, but the amount of tissue needed to produce 500 tubes will suffice to prepare about 50 plates.

It is clear that an enormous increase has been obtained in the amount of information provided by one animal. But, of course, the kind of information (in the nontechnical sense) is quite different, and completely appropriate to the system studied, for the virus particles can now be counted instead of being indirectly estimated. Finally, by such methods more than one kind of virus can be counted in one sample, owing to differences in appearance of the spots on the plate. Sanders concludes in general that tissue culture methods provide (in the nontechnical sense) more information, more precise information, and new kinds of information; he also predicts with confidence that this type of replacement will continue unabated. He ends with a final important aspect of tissue cultures which we have not hitherto noted--the maintenance of cell lines by transplantation *in vitro*, as in the case of the famous HeLa cell, isolated from human material in 1952, and since used all over the world in polio studies. By such means, the use of animals (apart from the original human or animal donor) is eliminated altogether, thus converting relative into absolute replacement. In fine, in Sander's words, "the animal virologist has great cause to rejoice at his liberation from the hazards and uncertainties of animal experiment. 'At this point'--to quote Alice in Wonderland--'One of the guinea-pigs cheered, and was removed by an officer of the court.'"

Other Uses of Tissue Culture and the Toxicity Problem

The uses of tissue culture (and other *in vitro* animal preparations) are far from exhausted by virology, though this is the only field in which it has been fully exploited. In carcinology, chemotherapy, pharmacology, bioassay, and toxicity testing, the method offers great advantages and has been developed to some extent, though its potentialities are far greater than its current usage. In hormone assay, for instance, two important purposes still largely employing whole animals are those of insulin assay and the assay of cortical steroids (Table 18). The former we have seen to be a priority for replacement; the latter involve, at the mildest, adrenalectomy. As long ago as 1923, Adberhalden and Gellhorn showed effects of directly applied insulin upon *in vitro* preparations of guinea pig small intestine and colon, rat small intestine, and frog esophagus. These effects, unlike those claimed by still earlier investigators, were not due to contamination by phenol (cf. Bachrach, 1953). It is also known that insulin increases glucose uptake by the isolated rat diaphragms of normal and alloxan diabetic rats, so the latter condition would be unnecessary (Beloff-Chain *et al,* 1955. The same preparation is influenced in certain chemical conditions by directly applied growth hormone (Randle and Whitney, 1957). As for the steroids, hydrocortisone, corticosterone, and adrenal extracts produce a direct cytotoxic action on rabbit lymphocytes *in vitro.* An excellent correlation has been reported between glycogen-depositing activity and this cytocidal assay (see review by Dorfman, 1954, who comments, "This method warrants further study for specificity and reproducibility; the

convenience and sensitivity appear to be excellent"). Such techniques seem to require further investigation. The great rewards reaped by the virologist should provide some incentive for workers in these other fields.

If progress in the bioassay field is not yet all it could be, the position is more serious in that of toxicity testing. This is one usage which is an urgent humanitarian problem, *both* numerically (Table 18) *and* in terms of severity, for it regularly involves a finite and large incidence of distress which is often considerable and sometimes acute. It is the avowed wish of workers in this field (cf. Bacharach, 1955a) to adopt replacement methods wherever possible in bioassay *sensu stricto,* alike on humane and economic grounds. A distinction is properly made between bioassay in the restricted sense, and the general problem of toxicity testing. Bioassay is the detection and quantitative estimation of a known activity or principle (sometimes a known molecule or molecular component) in a relatively impure preparation, usually though not invariably assessed by comparison with a preparation of standard purity and potency. Here any method which will regularly detect and estimate the activity in question is readily welcomed. Toxicity testing is sometimes more complex. In general, it may mean the assessment of various unknown or unpredictable special activities with the general property in common of toxicity to higher animals. As such, it is argued, toxicity testing must continue indefinitely to be practiced on mammals. The argument is a special (and the most important) case of the high-fidelity argument. It acquires its force from the importance rightly attached to the need for safeguarding human patients against toxic side effects of drugs (and also, we may surmise, from the irrational emotions associated with the concept of poison--and especially of poison administered in the guise of medicine--Russell and Russell, in press).

Some general considerations may clear the ground for a rational discussion of the toxicity problem. First (and this is often put forward in support of the use of mammals), what is important here is not an absolute quantity, as in bioassay, but a ratio. A very large number of substances are toxic in high enough dosage-some of the vitamins, for instance. The important concept, therefore, is the therapeutic index of a drug--that is, the ratio between its toxic and its therapeutically effective dosage. If this ratio is great, the drug or preparation is sage, since it allows for wide variations between human individual patients in sensitivity to the toxic effects.

This restriction is not so formidable as appears at first sight. The therapeutic dose ranges of many groups of biological substances are of similar orders of magnitude. We can, therefore, often tell that a preparation of given *absolute* toxic dosage will probably have too low a therapeutic index, and may be discarded as unsafe.

Toxicity is a function of two groups of variable--first, the actual effect of a substance on either general or specific systems of cellular metabolism, and second, the

mechanisms of excretion and detoxification in the human body which determine the actual amounts (rates, durations, etc.) to which the cells are exposed when a given dose is administered. The former property can readily be studied on isolated tissues or organs. The latter can only be studied in the whole organism. But the high-fidelity arguments cut both ways, for the mechanisms of excretion and detoxification in nonhuman mammal species frequently differ from ours. This objection is met in practice by erring on the side of caution, and by using more than one mammal species; it cannot ever be fully met, for there may always be metabolic peculiarities specific to man.

Toxicity testing, evidently, involves two stages--*cellular* and *organismic.* A very important distinction now arises in practice between two quite different kinds of toxicity test (probably confounded in our tables). First, there is the routine toxicity testing of well-known biological preparations in production. This is usually called *batch-testing* or *quality control,* and is an important process in the pharmaceutical industry. Once such a routine has continued long enough, it is little more than a test for the presence of a relatively constant group of impurities known to be associated with some batches of the preparation in question. The special nature of the toxic effects of these may by this time be well-known, and we may single out batch-testing as a special case where our proportionately greater knowledge should give us proportionately greater control of the procedures used. There is no reason why we should not here make use of the *correlation* principle, and research on these lines is at present being conducted under UFAW auspices. *In vitro* tests might be perfectly suitable, either through simple parallelism without causal understanding on our part, or by selection of models discriminative for the known impurities.

The second kind of toxicity test is the *screening* of new compounds and preparations for their toxic effects. This has assumed great importance now that organic chemists are annually producing vast numbers of new compounds. These have to be scanned for therapeutic effects, and this work, in fact, accounts for most of the applied research conducted in the big drug houses, which are acting as a giant filtration mechanism. All these compounds must also be screened for the toxic effects which would make them useless in practice. The scale of this work is considerable, especially in the United States, where the screening of ten thousand compounds in one laboratory is mentioned as a matter of course (Everett, 1956). Here we are much more in the dark. The *kind* of toxic effect which may arise is virtually unknown. Thus, in the report just cited, only ten of the ten thousand compounds produced one particular symptom in mice.

In theory, we should be able to classify all the ways in which toxic and lethal symptoms are produced in a higher animal. Toxicity may in general mean effects on the general metabolism of all cells or selective effects with special metabolic

characteristics. These two effects may be linked by such factors as sensitivity differences between cells to toxic effects on metabolic processes they have in common.

For both general and selective toxicity, an important principle is gradually coming into use--the principle of *scouting*. Thus Livingood and Hu (1954) found good correlation between toxic effects of drugs on tissue cultures and their irritant effects on human and rabbit skin. They proceed to discuss the use of tissue cultures for scout testing of new therapeutic agents in respect of potential capacity for causing irritation. The scout principle is simple. It means discarding any new drugs designed for particular purposes if their effects on tissue cultures are such as to give a poor prognosis for their effects on whole organisms and man. (Detoxicification does not arise in this particular instance, for the substances where intended for local application.) Nobody can object to the scout procedure on grounds of public safety, since it is merely a rapid and humane method of *discarding* compounds--a sort of prescreening.

Scout methods are specially to the point in carcinology, with its key problems of differential organ sensitivity and selective tissue destruction. Here the use of isolated tissues of specific organs or character becomes a very great advantage. In this connection, we may cite some comments made by Walpole (1957), in a discussion of "the contrast between the dramatic effects of some antitumor agents upon experimental tumors and the severe limitations to their usefulness, particularly against solid tumors, in man". Walpole considered the possibility of three types of selective toxicity, directed "respectively towards (a) all dividing cells, normal or neoplastic, within the body; (b) cells of or arising from one or other tissue; and (c) neoplastic cells as such". Available antitumor agents showed powerful selective toxicity of the first type. By means of this, he suggested, they inhibited growth of the rapidly growing animal tumors commonly used for screening, but not that of much slower growing solid neoplasms of man, at levels of dosage below those fatal for the more actively proliferating normal tissue. Tissue culture methods obviously provide excellent opportunities for clear-cut investigation of such problems, and as such are beginning to come into their own (cf. also Danielli, 1957). Carcinologists have to think in terms of special therapeutic indices of their own, for which, in both research and screening, tissue cultures supply excellent test material.

In fact, there has been in recent years a surprisingly abundant and purposeful literature on the existing and potential applications of tissue culture in pharmacology, carcinology and chemotherapeutics. We may, therefore, hope that great changes in large-scale commercial practice may be on the way, especially if the logistic difficulties are overcome under the spur of virological requirements. These changes might revolutionize bioassay, screening, and batch testing.

We need not attempt here to review this huge field, beyond calling attention to four key compilations. A whole volume of the Annals of the New York Academy of Sciences was devoted in 1952 to the uses of the chick embryo. A large section of the fourth volume *Methods in Medical Research* (Visscher, ed., 1951) was devoted to the uses of tissue culture in pharmacology, edited by Pomerat, an active worker in this field. (In another part of this same volume it is pleasing to learn that the study of gastric secretion is being increasingly carried on by means of *in vitro* preparations instead of the uncomfortable operations on whole mammals which formerly made this study directly inhumane--Davenport, 1951). Third and most important for reference purposes is the "extraordinarily comprehensive *Bibliography of Research in Tissue Culture,* compiled so painstakingly by Margaret R. Murray, and Gertrude Kopech" (Pomerat and Leake, 1954). In reviewing this great work, continue Pomerat and Leake,

"... one is impressed by the amazing number of references to drug action studied by tissue culture methods... On drug addiction studied in tissue culture there are listed 128 reports... excellent cross-referencing... makes it possible to find source material on the tissue culture effects of practically all types of drugs and poisons. Indeed, there are 57 pages of references to the poisonous action of chemicals as observed by tissue culture, totalling perhaps 1,800 publications."

Fourth, another valuable volume of the Annals of the New York Academy (58, 7, 1954) is devoted to *Tissue Culture Technique in Pharmacology.* One of the three parts of this volume concerns "Special Assay Techniques: Comparison Between *In Vitro* and *In Vivo* Results".

Extensive reference to these compilations is unnecessary here. Since we have made several references to digitalis, we may note a paper by Paff (1940) which supplies a workable assay method based on the contractile activity in explants of chick embryonic hearts (cited in Pomerat, 1951). Several of the papers in the three many-authored volumes raise general aspects of the use of tissue culture. For instance, such methods are said by Buchsbaum (1951) to

"... offer two main advantages over whole-animal material in studying the effects of any physical or chemical agent on biological material: (1) the advantage of dealing with a group of cells of one type (or even a single cell) in a medium which may be known and controlled; (2) the advantage of direct observation. Of course these advantages impose restrictions in drawing conclusions from experiments--the conclusions apply only to the experimental culture used. However, such conclusions may evolve hypotheses which may guide whole-body physiologic research."

This is a clear exposition of the scout principle in research itself.

In connection with assay and toxicity, the papers of Pomerat (1951) and Pomerat and Leake (1954) are of special importance, and may serve as primary sources to anyone interested in extending replacement in pharmacology. The second paper attempts wholesale quotation, and we may end this section by brief summary.

The authors list three disadvantages and five advantages of tissue culture methods in pharmacology. The advantages turn mainly on the absence of a host of control problems which arise when whole animals are used, and which will be conspicuous in our next chapter. From this balance sheet they conclude that tissue culture methods may be profitably used "for rapid screening of the abundance of new chemical agents which are continually being developed by organic chemists. This screening can be undertaken for such general matters as differential toxicity and specific organ susceptibility." It may be extended to "chemotherapeutic screening, where the parasitic organisms involved may be grown directly in the tissues concerned", an arrangement "well suited... for the systematic screening of new antibiotics". Variation in response to chemicals of the various organs of the mammalian body can be studies by separate culturing of these organs. Specific quantitative estimates of susceptibility obtained in this way are valuable "in learning in advance the possibility of unsatisfactory side actions of drugs that may be developed for some specific organ effect". Specific screening possibilities mentioned are those of antihistamines (on cultured human nasal mucous membrane), cardiac glucosides (digitalis, etc.), neurotropic drugs, drugs acting on the skin, and specific tissue nutritional factors. Some of the inviting prospects for pharmacological research are also exposed to view.

Finally a very comprehensive account is given of the toxicity to cultures of different tissues of a large number of drugs of all kinds, with elaborate tabulation and bibliography. From these results, the authors are able to suggest the most promising indications for tissue culture screening. One special observation may be mentioned. A certain group of preparations for local skin application had been deprived of the countenance of the Council on Pharmacy and Chemistry of the American Medical Association, and the basis of "producing cutaneous sensitivity". This harmful side effect could have been predicted from tissue culture results. It is abundantly clear from this paper and the other sources mentioned that replacement in screening methods is in no way prejudicial to public safety; and on that note, we may suitably close this section.

[1]ICLA is now taking up the general question of laboratory animal transport (Lane-Petter, 1958).
[2]The 'bit' (=binary digit) is a widely-used unit introduced into information theory by J.W. Tukey (Shannon and Weaver, 1949). It is the logarithm to the base 2 of the number of distinguishable members of a set of states. One animal in the example can take 2 distinguishable sates, and thus provides one bit. One hundred tubes can take

2^{100} states, and provide 100 bits. (Not all of this information is necessarily used in a given procedure.) The intuitive "rightness" of the logarithmic measurement of information, employed in the formulations of Fisher (1921), Shannon (l.c.) and Wiener and Von Neumann (Wiener, 1948), can be readily shown by reference to coin-tossing (Russell, in press, c). If we toss a coin once and tell you how it fell, we provide one bit. If we toss it twice, there could have been four (2^2) different results, so we have provided 2 bits. If we toss it three times, and report the result, we provide 3 bits. (In this case there would have been 8 [2^3] possible results.) For every additional toss, we provide one extra bit of information. The use of a *binary* digit was adopted because specially convenient for telephone and telegraph relay systems, and for use in connection with digital computers, which usually employ the binary scale.