Faults and Fallacies in Microbiology
The Fourth Marjory Stephenson Memorial Lecture

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I need hardly say how much I appreciate the honour of being asked to give the fourth lecture in memory of Marjory Stephenson and how little I feel able to do justice to the task. Though many of you here knew her better than I did, I may perhaps be allowed to claim a small share in the success which she achieved. In the early twenties, when she was brooding over problems of growth and nutrition, she realized the necessity of using a quantitative technique. For this reason she came to Manchester where Topley had recently inaugurated the Diploma in Bacteriology course, and it was there that I had the privilege of teaching her how to count bacteria.

The choice of a subject for this lecture proved very difficult. As I have done no bench work for 14 years, it had to be a general one. I have always been interested in technique, and therefore thought it might be worth studying the part played by faulty technique in reaching erroneous conclusions. I found an almost embarrassing richness of material before me, and I have perforce limited myself to a few illustrative examples.

Many of you will regard studying the errors of others as a presumptuous and invidious undertaking. I admit this. My purpose, however, is not, as that of some of the literary critics has been, to denigrate the outstanding men of the past, but to try and learn how lesser men can avoid falling into their errors. Even the greatest of scientists may go wrong, and all of us should realize the abyss at our side and say with John Bradford 'But for the grace of God there go I'. So far from attempting to act as a judge, I propose to be no more than an honest seeker after truth and of the ways of reaching it. I approach my subject, as every scientific investigator should, wearing 'the napless vesture of humility'.

Faulty technique

I shall start by quoting one example of the use of faulty technique which, though the best available at the time, has resulted in the perpetuation of error. In 1914 Brown, in India, made a careful study of the relation between the dry and moist weight of organisms, their numbers and their opacity. He was particularly impressed by the usefulness of opacity as a measure of the bacterial content of a suspension, and together with Kirwan (Brown & Kirwan, 1915) he introduced the following year a series of tubes labelled 1 to 10 made up with known amounts of a suspension of barium sulphate. These were very similar to those prepared by McFarland (see Kolmer & Boerner, 1941) in the United States of America. In 1924 Cunningham & Timothy published a series of tables...
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purporting to show the number of organisms of several different bacterial species corresponding to each of Brown & Kirwan's opacity tubes. This would have been a useful table if only the bacterial counts had been made by a reliable method. Unfortunately the method used by Cunningham & Timothy was to stain the organisms with carbol-fuchsin and to count them on a haemocytometer slide with a high-power objective. Since the average diameter of the organisms they were counting was less than 1 μ, since the depth of a Thoma-Zeiss chamber is 100 μ, and since the staining of organisms from a 24 hr. culture is very irregular, it is not difficult to understand why this method underestimated the total number of organisms alive and dead in any given suspension. When a more accurate method is used, namely that of the Helber slide examined under dark-ground illumination with a low-power objective and a compensating eyepiece (Wilson, 1922), it is found that the figures for Bacterium coli, for example, in Cunningham & Timothy's table are only about one-third of the real number. The result has been that many of the figures quoted in bacteriological literature during the past 30 years are unreliable. The mistake is aggravated by the fact that opacity is a measure of the total bacterial content of a suspension and not of the number of living organisms in it, so that when these figures are used for calculating the number of organisms in virulence experiments they are often grossly misleading (see Wilson, 1926). Moreover, the figures corresponding to McFarland's opacity scale used by the Americans do not agree with those corresponding to the Brown & Kirwan tubes used in this country. Because of this a great deal of confusion arose when some years ago we were trying to repeat the American observations on the prophylactic value of a pertussis vaccine, and found that the bacterial content of a given vaccine was estimated quite differently in the two countries. In fact, though our results were subject to criticism on the ground that we were using too small a dosage, comparison showed that the British vaccine contained at least three times as many bacteria as the American one (see McFarlan, Topley & Fisher, 1945).

I suggest that the World Health Organization might well consider the introduction of a standard method of counting which would do away with the present anomalies.

Failure to control one's technique

This is perhaps the commonest failure of all, and I could give you several examples of it. Let me start on the filtrable forms of the tubercle bacillus.

Filtrable forms of the tubercle bacillus

In 1910 Fontes, in Brazil, filtered some caseous pus through a Berkefeld candle and injected the filtrate subcutaneously into guinea-pigs. After a fortnight one of the animals showed a swelling of the inguinal glands; no acid-fast bacilli were visible microscopically. The spleen of this animal was inoculated into fresh guinea-pigs; when the animals were killed 5 months later acid-fast bacilli were found in the lymph nodes and lungs. These observations were confirmed by numerous workers. In general, it was observed
that when pure cultures of tubercle bacilli or tuberculous products were
ground up and filtered through a Berkefeld or Chamberland candle, and
the filtrate was injected in 1–10 ml. quantities subcutaneously into guinea-
pigs, a peculiar disease was liable to follow which differed widely from the usual
picture of experimental tuberculosis. The animals survived indefinitely or died
after 2–4 months. At necropsy there was no local lesion; one or more groups
of lymph nodes—particularly the tracheobronchial—were swollen but rarely
caseous; and at times small lesions were found in the spleen or lungs. Careful
examination with the microscope revealed a few acid-fast bacilli in the lymph
nodes or other lesions. When the nodes were injected into fresh guinea-pigs, the
same type of disease followed. The conclusion was drawn that the tubercle
bacillus passed through a developmental cycle, one stage of which was charac-
terized by the presence of minute filtrable forms capable of giving rise to a
modified form of tuberculosis in experimental animals. It was even supposed
by Calmette that these filtrable forms were able to pass through the placenta
and infect the foetus (Calmette, Valtis, Nègre & Boquet, 1925; Calmette,
Valtis & Lacomme, 1926).

This kind of work is beset with technical errors, and unless these are avoided
by suitably controlled observations all the way, they are liable to give rise, as
here, to unjustified conclusions. Porous earthenware filters cannot be properly
standardized and may occasionally let through the odd bacillus. The type of
tuberculosis that follows the inoculation of guinea-pigs with minimal numbers
of tubercle bacilli is one that progresses very slowly, causes little caseation in
the lymphatic nodes, and is characterized by healing of the proximal lesions
and gradual spread of the distal (Schwabacher & Wilson, 1937).

Acid-fast bacilli in the mediastinal and mesenteric lymph nodes are not
uncommon in healthy guinea-pigs as the result apparently of their inhalation
or ingestion in hay and straw. Caseous material, and particularly broth cultures,
may contain tuberculin which is responsible for the cachexia so often observed.
Moreover, the type of tuberculin that was used in these experiments to follow
the course of infection may contain acid-fast bacillary particles capable of
cauing confusion on microscopical examination. These are some of the errors
involved, and it is noteworthy that those workers who took care to avoid them
were unable to reproduce the lesions observed by Fontes and his followers.
I do not wish to deny the existence of a filtrable stage of the tubercle bacillus,
but I do wish to point out that the conclusion in favour of its supposed
existence might never have been drawn if the technique used to support it had
been properly controlled from the start.

Tuberculous bacilaemia

Another example of failure to take sufficient precautions against error is
provided by the long series of observations, culminating in those of Löwenstein
and his followers, on the occurrence of tuberculous bacilaemia. Pulmonary
tuberculosis is one of the few diseases in which a laboratory diagnosis can be
made with reasonable certainty by microscopical examination alone. For this
reason the examination of sputum and of other material of pathological origin
has often been left to persons with little or no bacteriological training, who were unaware of the numerous pitfalls that exist in this type of work. Tuberculosis has always been a hunting ground for the bacteriological tyro, and probably more errors have been promulgated in relation to this disease than to any other.

Many of the early workers reported the microscopical finding of tubercle bacilli in the blood of a high proportion of tuberculous patients and even of non-tuberculous persons and of patients suffering from other diseases. They did not appreciate the deceptive acid-fast artefacts that may be present in stained films of blood, particularly those derived from the lipid coat of the lysed red blood corpuscles. Nor did they take precautions against the contamination of their materials with the multiple saprophytic acid-fast bacilli that exist in dust, in reagents made up with old distilled water or with tap water, and in leather and rubber washers.

To confirm their claims, a number of these workers carried out animal inoculation. Ever since the time of Robert Koch this method has been recognized as the most delicate of all methods for demonstrating the presence of tubercle bacilli, so that positive results by guinea-pig inoculation are naturally regarded as being conclusive.

It came as a great shock to me, when some years ago I was called upon to investigate these claims, to find that many of those who had used this method had completely travestied its whole purpose. Instead of using it to show that the bacillus in question was able to set up a progressive, generalized, and fatal disease in the guinea-pig, characterized by the development of lesions typical in their appearance and distribution and containing acid-fast bacilli, they had contented themselves with diagnosing experimental tuberculosis by finding acid-fast bacilli in the spleen in the complete absence of macroscopic lesions or by relying entirely on a positive tuberculin reaction. The occurrence of natural tuberculosis and of other diseases simulating tuberculosis in some respects, caused by organisms of the Pasteurella, Brucella and Salmonella groups, provided an added stumbling block.

The extravagant claims made by some of the early workers on the frequency of tuberculous bacillaemia appeared to receive abundant confirmation by Löwenstein and his colleagues (for references see Wilson, 1933) at Vienna in the early thirties of this century. Löwenstein devised an excellent egg medium for the growth of tubercle bacilli, and by its means was able to report the isolation of these organisms from the blood of patients in the early stages of both pulmonary and non-pulmonary tuberculosis. Passing on to other diseases, Löwenstein and his followers claimed success in demonstrating the presence of tubercle bacilli in the blood of patients suffering from various forms of skin disease, from polyarthritis, from chorea, rheumatism, multiple sclerosis, retrobulbar neuritis, schizophrenia, polycythaemia rubra, endocarditis and tenosynovitis.

What was the explanation of results that common sense alone would consider ridiculous? There were many reasons which I have no time to describe in full. Two of the principal ones were that: (a) cultures were considered
positive when, in the absence of visible colony formation, smears from the surface of the medium showed acid-fast bacilli on microscopical examination; and (b) smears were often made on slides which had been used previously for tuberculous specimens stained by Ziehl–Neelsen. Some of the macroscopic cultures that were obtained were almost certainly cultures of saprophytic acid-fast bacilli from the environment and not of true tubercle bacilli.

Critical examination of the results obtained by workers who exacted a high standard of technique and interpretation shows that tuberculous bacillaemia is seldom demonstrable during life, except in generalized tuberculosis or during an advanced stage of pulmonary tuberculosis. To reach this conclusion, however, and to refute the claims of Löwenstein and his associates to have provided a method for the early diagnosis of tuberculosis necessitated the devotion of a large amount of work by over a hundred observers in different parts of the world. The claim was of such tremendous clinical importance that it could not be neglected, and in consequence a great deal of time that might have been devoted to a better cause was wasted.

It fell to me to draw up a critical appreciation of this work for the Medical Research Council, and I shall never forget the late Sir Walter Fletcher’s remark when I went down to Old Queen Street to discuss the report with him personally. He said: ‘You know, Wilson, I am a physiologist, and I know that unless a physiologist uses a good technique he gets no results; but it seems to me that in bacteriology the worse your technique is, the more astounding are your results.’

Supposed cultivation of viruses from poliomyelitis, influenza, and numerous other diseases

One of the most extraordinary chapters in the history of bacteriology concerns the supposed isolation of filtrable organisms from a variety of diseases by means of the ascitic fluid kidney culture technique. It is all the more extraordinary in that some of the foremost bacteriologists in the world, such as Simon Flexner and Hideyo Noguchi, were among those who fell into error.

In 1911 Noguchi introduced a medium for the cultivation of spirochaetes. It was a modification of Schereschewsky’s (1909) medium and consisted essentially of 15–20 ml. of sterile unfiltered serum or ascitic fluid contained in a tall test-tube. On the recommendation of Theobald Smith a small piece of freshly excised sterile rabbit kidney was added to each tube. The medium was covered with a layer of liquid paraffin 1–2 cm. deep to shut out the air. Sometimes agar was included. Noguchi had considerable success with this medium and, though few workers now would be prepared to agree with his claim to have cultivated virulent Treponema pallidum (1911), he did succeed in cultivating a number of other spirochaetes (Noguchi, 1912a, b, c). This medium apparently owed its virtue to the natural animal protein provided by the serum or ascitic fluid and to the anaerobic conditions prevailing around the fragment of kidney.

Encouraged by his success, Noguchi (1918) turned his attention to other organisms that had not yet been cultivated. The first one he chose was the parasite of rabies. He inoculated his medium with a fragment from the central
nervous system of a rabid animal, and described how during the following days
an opalescent haze appeared around the tissue. Microscopical examination
revealed the presence of what appeared to be pleomorphic, often nucleated,
particles, staining with Giemsa, and ranging in size from the merest granules
to multinucleated bodies 12µ in diameter. Many of these bodies bore a close
resemblance to Negri corpuscles, as was evident from photomicrographs pro-
vided for comparison. Noguchi was able to transfer the supposed parasite to
fresh tubes of medium and, by inoculating cultures containing the granular,
pleomorphic or nucleated bodies into animals, to reproduce the disease in dogs,
rabbits and guinea-pigs. Film preparations from the brain of the animals con-
tained the granular and sometimes the nucleated bodies in large numbers.
Noguchi regarded the organism as probably a protozoon.

Closely following this work came a description by Flexner & Noguchi (1913)
of the cultivation of the organism of poliomyelitis. The same technique was
used, and the same type of turbidity developed around the tissues. Some-
times culture was successful when the inoculum consisted of a suspension of
brain filtered through a Berkefeld V candle instead of a fragment of tissue.
When agar was incorporated in the medium minute colonies could be seen
extending up to within 2 cm. or so of the surface. Microscopical examination
revealed the presence of minute globoid bodies, 0.15–0.3µ in diameter, arranged
in pairs, short chains, and small masses, staining by Giemsa and tending to be
Gram-negative when young and Gram-positive when old. Several experiments
were performed in which monkeys are said to have developed poliomyelitis
after intracerebral or intrasciatic injection with third- or fifth-generation
cultures as well as with brain from passage monkeys. Not all the inoculated
animals developed the disease, but in those which did typical histological
findings were reported in the central nervous system. Moreover, by a special
staining technique globoid bodies were found in smears and sections of the
nervous tissue.

The article in which this work is reported is a long one; the monkey inocula-
tions are described individually; numerous photographs and photomicrographs
are provided; and it is written by experienced workers from the Rockefeller
Institute who were aware of most of the pitfalls. Apart from the absence of
certain desirable controls, the article could hardly help but carry conviction to
the unbiased reader.

After the apparent success with this medium in the isolation of the causative
agents of rabies and poliomyelitis, Foster (1917), a major in the Medical Corps
of the United States Army, described the cultivation of a filtrable virus from
the common cold. Both the macroscopic and the microscopic appearances in
the cultures were very similar to those reported by Flexner & Noguchi for
poliomyelitis, but the globoid bodies were more variable in size and the larger
ones among them showed signs of budding. Subculture into ascitic fluid
containing agar resulted in the development of minute colonies around the
kidney at the bottom of the tube. Second-generation cultures in fluid medium
filtered through a Berkefeld N candle and instilled into the nose gave rise to
colds in 11 out of 11 human volunteers, and from 7 of them the virus was
recovered in culture. Much the same findings were recorded in the same year by Dold (1917) working at Shanghai, though Dold was unable to see anything distinctive in the microscopical picture.

It is not surprising that, at a time when the findings of Flexner & Noguchi were credited with the full weight of authority that lay behind them, workers should look for other diseases to which to apply the new method.

At the 20th General Hospital of the British Expeditionary Force in the area of Étaples, Bradford, Bashford & J. A. Wilson (1918–19a), after an apparent preliminary success in isolating the aetiological agent of acute infective polynephritis, turned their attention to the great problem of influenza. They investigated cases of the disease during the severe outbreaks in the autumn of 1918 and the early spring of 1919 (1918–19b). Working in the strictest secrecy, surrounded by an armed guard, and out of contact with all colleagues, J. A. Wilson recorded the isolation of the influenza virus in Noguchi medium from 22 out of 25 samples of blood, 14 out of 16 pleural fluids, 40 out of 40 sputa, as well as from odd specimens of bile, urine, and cerebrospinal fluid, while Bashford reproduced the disease with cultures in guinea-pigs, rabbits and monkeys. Subcutaneous injection was usually without effect, but intravenous and subdural inoculations were almost invariably followed by the disease. The virus was recovered from the inoculated animals.

Not content with this achievement, Bradford, Bashford & Wilson (1919) applied the same technique to other diseases, and were soon able to record the presence of filter-passing organisms in material from cases of trench fever, acute nephritis, measles, rubella, mumps, typhus and encephalitis lethargica. The latter part of this work was transferred from Étaples to the Lister Institute, and was regarded as so important that demobilization of the laboratory assistants was voluntarily delayed in order to allow of its completion.

It was time for the bubble to be pricked. Already Kraus & Barbará (1914) in Germany, attempting to repeat Noguchi's findings in rabies, had been able to confirm the presence of pleomorphic chromatic corpuscles and granules in cultures, but had pointed out that apparently identical bodies were present in normal ascitic fluid. Moreover, they had failed to reproduce the disease in animals by means of cultures. Probably because of the war this important paper was overlooked, and it was left to Arkwright (1919) to bring the whole unhappy tale to an end. Examining Wilson's cultures, he found that many of them were contaminated with staphylococci and diphtheroid bacilli. These organisms often stained only in the centre or at the poles and consequently appeared like minute globoid bodies. In cultures that were sterile he observed the presence of smaller or larger granules, 0.2–0.3 μ in diameter, globoid in shape or bipolar-stained, and showed that these consisted not of viruses but of particles of protein precipitated by the acid resulting from autolysis of the piece of kidney at the bottom of the tube. Discussing these results, Arkwright attributed the mistakes that had been made to working in isolation, to failure to exclude contamination by subculture on to ordinary media, to undue reliance on the efficacy of porcelain filters for removing
bacteria, and to inadequate staining. It is only fair to add that at the end of
Arkwright's paper Bradford and Wilson withdrew their claims.

From a careful study of the papers in which work with Noguchi's medium is
reported, I find it hard to explain many of the results, particularly those
following the inoculation of cultures into animals. The difficulty of inter-
preting the appearances in stained films from fluid cultures in which the
protein is precipitated by heating is understandable, and all the more so when
the medium contains blood or serum and has been incubated for 5 days or
more till a deposit has formed. That a variety of pleomorphic bodies should be
seen under such conditions is not surprising, but it is rather surprising that
they were said to have been absent from control cultures.

The risk of contamination in putting up Noguchi tubes hardly needs em-
phasizing. Each tube has to be opened at least five times before the culture is
ready for incubation. Arkwright, who put up a series of tubes himself using
material from trench fever, found that 20% were contaminated—a proportion
substantially the same as that of control tubes, namely 22%.

The technique of filtration used by Wilson was crude. He obtained his
negative pressure by attaching his Berkefeld or Massen candles to a cooling
autoclave and filtered for 1–4 hr. It is not to be wondered at that in these
circumstances some micro-organisms were sucked through.

The pathological appearances produced by the intravenous or subdural
injection of cultures into animals and believed by Bashford to be characteristic
of influenza may at times have been due to staphylococci, particularly as
nephritis was often recorded on post-mortem examination. Dark patches and
haemorrhages are not uncommon in the lungs of guinea-pigs killed by various
means in the laboratory, and had more attention been concentrated on in-
jections with control cultures the pathological appearances noted might have
been interpreted more correctly.

In their work on poliomyelitis Flexner & Noguchi put up control tubes
consisting of kidney and ascitic fluid, or of infected brain and ascitic fluid, but
not tubes containing ascitic fluid, kidney and normal brain. Similarly, in their
experimental work on animals, they did not inject cultures made with normal
brain tissue. Nevertheless, it is not easy to understand why Flexner in
particular should have gone wrong in claiming to have reproduced polio-
myelitis by the injection of cultures containing globoid bodies. Working with
Lewis in 1910, Flexner had reproduced the disease in monkeys with human
material, and had carried it over from monkey to monkey by subdural
injection of cord suspensions. He had had, therefore, a considerable experi-
ence of the experimental disease, and yet in his work with Noguchi he must have
been mistaken. The diagnosis, however, of experimental poliomyelitis in
monkeys is by no means straightforward, and here again it is clear from Sabin's
(1959) recent article that the technique used is all-important.

It is well to realize that probably most of the erroneous conclusions based on
the use of Noguchi's medium would have been avoided if only a full set, instead
of a partial set, of controls had been systematically used throughout the
investigations.
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The danger of working in isolation referred to by Arkwright needs stressing. It is not only complete isolation that should be avoided, but relative isolation as well. Lone workers in the provinces, the colonies, and sparsely populated lands are not exposed to that healthy criticism which most workers in large towns enjoy. If there is a fault in their technique, there is no one to point it out to them. If their erroneous conclusions or ideas are not challenged at the start, they are liable to become fixed. When eventually they are challenged they have become so deeply implanted that their attempted refutation is often regarded as a personal insult, and is accompanied by an emotional reaction that is quite foreign to workers who are used to free discussion with their colleagues. Though, as we have seen, big institutes are not immune from error, research carried out in isolation is especially perilous, and no one who is wise will publish a discovery of any moment without seeking the opinion of one or two trusted experts in the field.

Confusion of natural and experimental disease

Confusion may be caused not only by the lesions of a natural disease but by latent infection in apparently healthy animals. This was responsible for some of the statements in the early German literature on food poisoning that carcass meat which appeared to be perfectly good on inspection nevertheless often contained organisms of the paratyphoid-Gärnter group. Mühlen, Dahm & Fürst (1909), for example, fed white mice on uncooked, pickled, and smoked meat that was apparently sound and found that over 50% of the animals died. From a considerable proportion of the dead mice Gärnter's bacillus was isolated. Direct culture tests on the meat were negative. This led the authors to conclude that the organisms must have been present in the meat but in only very small numbers.

It was not long before other workers in Germany pointed out the fallacy of this conclusion. Mice are subject to natural infection with Gärnter's bacillus, and the injection of foreign material may stimulate a latent infection into activity. The same fallacy underlay Nocard's (1898; see 1897) conclusion that psittacosis was caused by *Salmonella typhimurium* and M'Gowan's (1911) and Ferry's (1911) conclusion that dog distemper was caused by *Haemophilus bronchisepticus*.

It is sometimes difficult to know for certain whether an organism isolated from an animal was present in the injected material or was derived from the animal's own tissues. Errors due to this cause can generally be avoided when the organism in question is known to be a potential parasite of the animal that is under test. Difficulty arises, however, when the animal inoculation method is used to search for an organism whose identity is unknown. The virologists have been particularly troubled in this type of work. Francis & Magill (1938), for example, isolated a meningo-pneumonitis virus from ferrets that had been inoculated with throat washings of human patients suffering from an epidemic influenza-like disease. Whether the virus was derived from the patients or from the ferrets it was, however, impossible to say. The difficulty is increased by the tendency of some animal parasites that cause latent infections to be practically
avirulent and hence almost impossible to demonstrate. This is true of the mouse pneumonitis virus described by Horsfall & Hahn (1940). Under ordinary conditions it appears to be avirulent, but by serial passage through mice inoculated intranasally with infective lung it becomes capable of causing a disease characterized by pulmonary consolidation. The encephalomyelitis virus of mice described by Theiler (1937) is another virus that is sometimes so widely spread in mouse colonies that by the time the animals are a month old practically every one of them has been infected. The infection remains latent throughout life, but may be stirred up by the injection of foreign material. It is specially liable to cause confusion to workers looking for neurotropic viruses.

In this connexion it is interesting to note that the existence of a latent infection with one organism may enhance the virulence of a different organism. Gledhill & Niven (1957), for example, found that mice infected with *Eperythrozoon coccoides* were more sensitive than normal mice to the injection of filtered or formolized cultures of *Salmonella typhimurium*. This observation was of peculiar interest to me, because it made me wonder whether it might have a bearing on the results obtained by Topley and his co-workers in their studies on the epidemiology of mouse typhoid. In some of my own work, which I never published, I observed striking differences from month to month in the mortality of mice injected with the same dose of the same strain of *S. typhimurium*. Similar differences, though not necessarily concurrent, were observed in mice injected with a lipopolysaccharide fraction extracted from the strain at the beginning of the work. The mice were in groups of 80, and were injected once a month over a period of 5 years. As far as possible all normal variables were controlled. The differences observed were not seasonal in the ordinary sense, because periods of high mortality might occur at any time of the year, nor could any regularity be discerned in the curves formed by plotting the death rates. Neither Topley nor I at the time had considered the possibility of a latent bartonella or eperythrozoal infection. The mice were bought from different dealers and, though free from salmonella infection, may quite easily have been infected with one of these blood parasites. It is a matter for speculation to what extent such an infection may have been responsible for the irregular results obtained. Certainly in any future work on the epidemiology of a disease such as mouse typhoid care would have to be taken to exclude as far as possible the presence of latent infections with other organisms.

Confusion may occur not only between natural and experimental disease in animals, but between natural diseases occurring in a given species of animal or in man. A good example of this was the failure of the clinicians to distinguish between yellow fever and Weil's disease that led Noguchi to identify *Leptospira icteroides* as the cause of yellow fever (see Noguchi, 1925). In dogs the difficulty in differential diagnosis between the encephalitic manifestations of distemper, of hard pad disease, and of so-called hysteria has set laboratory workers a problem of great complexity in deciding whether one virus is responsible or whether two or more are concerned.

Generally speaking, it is advisable in the investigation of any disease to
define it clinically and epidemiologically before serious work on it is commenced in the laboratory. This may be a counsel of perfection, and admittedly bacteriological or virological examination may be necessary to reveal the difference between two clinically similar diseases of different aetiology. But failure in the past has at times resulted from neglect to study the epidemiological behaviour of a given disease with the same care as its clinical manifestations. It took, for instance, many years of epidemiological study to show that infectious hepatitis and serum hepatitis, though clinically alike, nevertheless behaved in their incidence, their mode of spread, and their infectivity as separate diseases. Once this was realized, and once it was appreciated that arsenic jaundice, homologous serum jaundice, and transfusion jaundice were all different expressions of one and the same disease, namely serum hepatitis, the interpretation of the laboratory findings, though still not definitive, was made very much easier.

Failure to appreciate the complexity of the situation

Though this hardly comes within the definition of technique, it is a mistake that has been frequently made by both medical and non-medical microbiologists. It is inappropriate to refer to it to-day because, as Woods (1953) pointed out in his inaugural lecture, it was Marjory Stephenson, among others, who realized the danger of drawing conclusions from the complex picture generally presented by Nature, and who indicated the ways in which progressive simplification might be effected in order to render the role of individual organisms more amenable to study. It would be almost equally apposite to stress the opposite danger, namely that of generalizing on the basis of observations made in too simple an environment. Biology can progress only by using both inductive and deductive reasoning, and it is when one of these is used to the exclusion of the other that false conclusions are likely to be reached.

One of the earliest examples is Metchnikoff’s hypothesis of intoxication resulting from the absorption of poisonous products generated by the proteolytic flora of the human intestine. Under the influence of Pasteur the fermentative activity of the intestinal bacteria had been regarded as assisting in digestion of the food taken in by the host. Metchnikoff (see Metchnikoff, 1921; Schieblich, 1929), on the other hand, who was concerned with the question of longevity, came to regard the products of putrefaction as responsible for the degenerative processes in the heart, arteries, and other essential structures of the body that led to a premature end of human existence. To combat this effect he proposed to replace the proteolytic organisms of the intestine by a lactobacillary flora. This he did by the ingestion of fermented milk, such as the Bulgarian yoghurt, containing organisms that broke down the lactose to lactic and other fatty acids. The fact that Metchnikoff himself died at the age of 71 is hardly a sufficient proof of the fallacy of his thesis, but subsequent experience on a wider scale has failed to substantiate it in any way. It has proved impossible to establish a lactobacillary flora in the adult human gut without the ingestion of impossibly large quantities of fermented milk, or of lactose or dextrin. Moreover in animals, and probably also in man, it has been
found that the natural intestinal bacteria lead to the synthesis of amino acids and of vitamins that play an important part in the nutrition of the host. As the recent work on the growth-promoting powers of chlortetracycline in farm animals has shown, the subject is, however, very complex, and the problems presented will take many years of study to resolve.

Other examples that might be quoted of over-simplification are the attempt to reproduce the defence system of the human body by mixtures of blood and organisms in a capillary tube, the danger of arguing by analogy from animals to man, of which I could quote many instances, and the evaluation of a disinfectant in practice by its performance under very simple and artificial conditions in the laboratory. The hope, too, that the cholera bacteriophage would behave in the complex environment of the human intestine in the same way as it did in a pure culture of cholera vibrios in the test-tube may perhaps be regarded as a further instance of the danger of over-simplification. It is not, of course, that any of these attempts was wrong in itself. In making them the authors were merely doing what Marjory Stephenson would undoubtedly have approved of. It is because far-reaching conclusions were drawn from them not justified by the evidence that I refer to the danger of over-simplification.

One of the most fertile reactions against this danger was Sir Henry Dale's contention that the standardization of biological products could not be undertaken by the same methods as those used for chemical substances. It was this that led to the development of the present world-wide organization for the control of biological products, and helped also to introduce the statistical method of approach into so many of the problems of microbiology and immunology.

Topley likewise reacted against over-simplification. At the time he started his main work towards the close of the First World War the literature was full of statements on the virulence of organisms, and particularly on their change in virulence as the result of some natural or artificial procedure, which was based on experiments made on minimal numbers of animals. Topley soon realized that the reaction of a given animal to a particular challenge was determined by a number of different factors, and that to attempt to draw far-reaching conclusions without taking account of the natural variation among animals belonging to a given group was to present a grossly over-simplified and often erroneous picture of reality. By enlisting the co-operation of Greenwood he was able to study the limits of natural variation and to plan his experiments in such a way as to make reasonably sure by virtue of the number of animals used that the answer obtained would have at least a certain degree of probability.

The importation of the statistical method by Dale and his followers into the realm of biological standardization and by Topley and his followers into the realm of immunology has profoundly affected the whole outlook of clinical and laboratory medicine, and has made possible the numerous field trials and investigations in which this country has excelled of recent years. The statistical method provides a compromise between the over-simplified environment of the test-tube and the uncontrolled complexity of the natural environment. So
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successful has its application been that, unless its limitations are appreciated, it may lead to the substitution of quantity for quality. In spite of its success, its use is to some extent a confession of failure—failure to make the perfect observation; and it should not be forgotten that the value of the statistical method is in proportion to the quality of the observations to which it is applied. It should never be offered as an excuse for laziness.

Conclusions

There are many other faults and fallacies which I might describe if time permitted, such as the mistakes that have been made in wrongly identifying the cause of a disease by failure to conform to Koch's postulates; overemphasis on the numbers rather than the nature of the organisms in assessing the quality of food substances; the unjustifiable transference of a generalization that is true in one department of microbiology to another department in which the premisses on which the generalization rests do not hold, such as the significance of *Escherichia coli* as an indicator of human excretal pollution; and in the field of epidemiology the drawing of general conclusions for a whole country from observations based on only limited parts of it.

The few examples I have quoted may, however, suffice to show how closely fact and fancy are woven into the tissue of scientific investigation. The younger workers who begin where the older ones leave off do not realize how much error has been perpetrated in arriving at the corpus of truth with which they start. Nearly all the errors of which I have spoken have arisen from imperfect technique and absence of full controls, or from over-simplification through failure to appreciate the complexity of the situation.

The conclusion I reach is that the scientific investigator should receive a wide general training, including the historical background of his subject, together with particular instruction in experimental technique and the errors of different techniques. The late Lord Horder used to say that every medical student should study elementary logic. This is good advice, but I should prefer to replace elementary logic by elementary statistics. Logic is excellent in its own sphere, but when applied to biology it may lead to curious results. The statistical approach is a better one, because it takes account of the concept of variation.

Another conclusion I would draw is the desirability of implanting a healthy scepticism, though not disrespect, of authority. Great men often make great mistakes, and microbiologists are no exception. Conversely, it is probably true to say that no fallacy is ever propagated without the support of some great man behind it. Whenever objective evidence is obtainable it is our duty to study that rather than to accept uncritically the conclusions that have been drawn from it by others. When, of course, objectivity cannot be obtained, as in the measurement of beauty and goodness and in the realm of religion, then tradition, authority and our own personal experience must serve as our guide.

Many people, such for example as Beveridge (1950), have tried to define the qualities that make a good research worker. I have tried to do the opposite, and examine the reasons for the production of bad research work. You have
listened patiently to me, patiently to what may well be considered a series of dull platitudes, and as I finish I expect you will heave a sigh of relief and say with Hamlet 'These tedious old fools'.

REFERENCES


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WILSON, G. S. (1926). The proportion of viable bacilli in agar cultures of *B. aertrycke* (Mutton), with special reference to the change in size of the organisms during growth, and in the opacity to which they give rise. *J. Hyg., Camb.* 25, 150.


*(Delivered before the Society for General Microbiology at its Twenty-eighth Meeting, 7 April 1959)*