ROUTINE AND CLINICAL LABORATORY RESEARCH.*

By W. R. O'Farrell.

IN selecting a subject for his inaugural address I understand it has been the custom in the past that the President should choose a theme which is of general interest and will, at the same time, contain, if possible, particular reference to the problems of the moment. During the coming year it is likely that one of the most interesting problems which will have to be faced by this Academy is the matter of research.

The fact that in Ireland facilities for organised research are badly needed has come to be fully recognised; funds have now become available, and the mechanism for carrying out a scheme is in the process of formation. When the preliminaries have been decided upon, the immediate problem will be to determine how the scheme can be most profitably applied, and what lines of research will be feasible under present conditions.

Hitherto research in Ireland has been a matter of private enterprise or has been largely limited to the various teaching bodies who have been seriously handicapped through lack of funds.

Even when the limited grants of the Medical Research Council ceased nothing was done to replace them.

Unlike workers in other countries where systematic research has been in operation for many years, we in Ireland will have to consider our position carefully and to proceed with caution if we are to ensure the ultimate success of the enterprise. When the scheme has been in operation for some time it will probably develop automatically, but it is evident that the first few years will be years of difficulty and anxiety. Before proceeding further it would be well to consider what is meant by research.

I would define research as a record of diligent study and investigation based upon sufficient material and embodying an intelligent interpretation of results.

That the product of such work should be successful in a commercial sense is desirable, but this should not be regarded as essential. Diligent and systematic research work, accurately recorded, is the establishment of truth and fact, and such facts may in themselves contain a practical value which may not be appreciated until after many years.

Research is a subject which appeals to everyone; indeed the average person has an inclination to suggest his particular fancy without having given due consideration to its practicability as a research subject. If, for example, he were asked to detail upon paper a complete list of the work which has already been done

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on the subject of his choice, the lines upon which he considers further work could be carried out, and, at the same time the outline of a plan for the carrying out of the work under present conditions he would perhaps not be so hasty in his suggestion.

Subjects for research may be advocated upon two grounds:—

(1) Based upon a brilliantly conceived idea.

(2) Based upon ideas resulting from experience of routine work.

I do not pretend to be able to suggest subjects based upon brilliantly conceived ideas, but I do hold that routine and research are closely interdependent. Routine supplies subjects for research and the results of research provide mechanism for the improvement of routine.

At the risk of boring you, I propose to review some of my experiences in various laboratories and to bring before you certain problems which have occurred to me in the course of routine work and which might possibly offer suitable fields for research; also to draw certain general inferences as to some of the facilities which are necessary for clinical research work from the laboratory point of view.

I have been fortunate in that I have worked in about a dozen laboratories in various parts of the world. Some of these laboratories operated under difficulties of place and climate, and others in more auspicious circumstances. There was one factor, however, common to all, viz., that the men with whom I had occasion to work were enthusiasts, and many of their names have come to be regarded since as household words in the world of scientific medicine.

Although the nature of my employment in the Royal Army Medical Corps tended to some instability, in that I was moved about from place to place, some account of my experiences may be of interest to those who have been in the more fortunate position of being stabilised in one country. It is possible at least that I may be able to bring the subject before you from an unfamiliar angle.

To avoid repetition I shall give approximate dates, denoting a change of station and at times a change of country. To some laboratories I was reposted after the lapse of years; in their case I shall note a few of the additional routine methods resulting from new discoveries which had been introduced during my absence.

There will be certain lapses in the record, denoting periods when enforced separation from pathology took place.

Prior to 1905, the then existing teaching universities and research institutions had laboratories which compare favourably with those of to-day. The smaller hospitals, however, had little laboratory space or equipment. There the work usually devolved on a junior member of the staff, who had neither the intention, nor indeed the inducement, to continue at pathology. A better clinician may have resulted from such an arrangement, but a

good pathologist was possibly lost. One may fairly say that clinical pathology offered no means of livelihood to the worker who was not at the same time attached to a university. These factors, of which the basis was financial inadequacy, seriously militated against the advance of clinical pathology, and laid the foundations of the insufficiency of personnel with which we in Dublin find ourselves faced to-day. Nevertheless, in these days, histological technique was excellent; sections, though perhaps not so large, but almost as good as those of the present day, could be cut and stained; the diagnosis of diphtheria and typhoid could be established and confirmed; blood counts could be carried out; sugar and urea could be estimated quantitatively in the urine.

In 1907, the new Royal Army Medical College at Millbank was completed. Arising indirectly out of the invaluable researches and discoveries of Wright, Bruce, and Leishman, in bacteriology and protozoology, and Firth's work in hygiene, the College was furnished with model bacteriological, vaccine and hygiene departments and possessed a good library. Colonel Leishman was one of the professors, and the departments were very efficiently staffed.

Apart from actual research work, the functions of the staff were to a large extent instructional. The junior, senior and specialist courses in bacteriology, tropical medicine and hygiene were almost perfect studies of theory and practice. Qualified workers and laboratory assistants were trained for home and overseas work. The College was in close connection with the Queen Alexandra Military Hospital, but was not attached to it, the hospital having its own laboratory.

A few of the laboratory procedures in use may be mentioned: blood cultures were employed for the diagnosis of pyogenic, typhoid, and Malta fever infections, but the general withdrawal of venous blood had not become fashionable. The various " Widal " reactions were almost entirely done by microscopic or micro-sedimentation methods. Blood counts had not become a widely used routine method of investigation, but the total leucocyte count was extensively employed. Prophylactic immunisation had been extensively used, but autogenous vaccines were not generally prepared by individual pathologists, it being considered necessary to control treatment by opsonic methods; this procedure and the time necessary for its performance, limited the universal application of vaccines in treatment.

1909. The Venereal Hospital at Rochester Row had recently been rebuilt. Colonel Lambkin (of mercurial cream fame) was in charge. About this time the earlier arsenical compounds were replacing the mercurial creams.

The hospital had a small room as a laboratory, but there was no pathologist. Nevertheless advantage was taken of the recent discovery by Schaudinn (1905) of the *spirochæta pallidum*, and the dark-ground illumination method was used as a routine for the early diagnosis of syphilis. Venereal disease, especially

gonococcal infection, occasioned considerable loss of efficiency and expense. Much was done, notably by Colonel Harrison, but the treatment of the disease was then, as it is to-day, a subject which required investigation.

In 1910, I was transferred to the Military Hospital, The Citadel, Cairo. As was the rule in the larger military hospitals, this hospital was equipped with a small laboratory. The pathologist, in addition to laboratory work, had other duties to perform, but there was a trained laboratory assistant. The work of the laboratory was routine. Macroscopic " Widal " reactions, using living bacillary suspensions without incubation, were attempted, but as only small quantities of serum were submitted only low dilutions could be prepared and the method was abandoned. Although the difference between the *entamœba histolytica* and the *entamœba coli* had been established by Schaudinn in 1903, and Shiga had discovered the organism of bacillary dysentery in 1898, dysentery had not as yet received much routine attention in this laboratory. No attempt was made to differentiate between the amœbic and bacillary varieties. This may have been partly due to lack of material, or to my unfamiliarity with the practical aspects of the work.

It is useful to recall here that Shiga, following up a suggestion made by Kitasato, searched for micro-organisms in the stools of dysentery patients which would specifically agglutinate with the serum of these patients, and that his efforts were crowned with success. The manner in which Shiga made his discovery furnishes an instructive example of the successful application of the then modern methods to etiological investigation. As a junior officer numerous duties devolved on me: one was the direction of venereal treatment, and part of the work consisted in identifying sources of infection. In a number of instances several individuals had exposed themselves to a single source of infection, although only one became infected. This might have been explained by minute trauma, but the question arose as to whether there was an underlying immunity factor at work.

From 1912 to 1916, I was attached to the Wellcome Tropical Research Laboratories, in Khartoum. For a number of years there had been great activity in the study of and in the advance of knowledge in tropical medicine. Amongst a few of the recent discoveries were the tick transmission of relapsing fever, the route by which the *ankylostoma duodenale* gains access to the intestine and the recognition of the *trypanosoma rhodesiense*. About this time " 606 " could be obtained in small quantities through the kindness of Professor Ehrlich, and was at that time administered, suspended in olive oil, by intramuscular injection.

The field of tropical research was pregnant with possibilities and routine work became necessarily associated with research. The possibility existed of bringing to light some parasite or some stage in the life history of a parasite hitherto unsuspected or unknown.

The Laboratories, which were directed by the late Andrew Balfour and A. J. Chalmers, were well equipped with pathological bacteriological, chemical and entomological departments; there was also a good library. The housing of these departments in one building was of the greatest mutual advantage and convenience. In contrast, the distance and dissociation from the Civil Hospital was a drawback.

The work of the laboratories was essentially research, and has appeared in various reports and journals. Some of this was of a comparative nature, such as researches on the " spirochætosis of Sudanese fowls." While examining cattle ticks, proposed to be used in spirochæte experiments, a new flagellate (*crithidia hyalommæ*) infecting the tick (*hyalommæ Aegyptium*) was discovered, and a cycle in its life history was studied. Human infections with treponemata and spirochætes were eagerly sought for, and numerous investigations were carried out on the treponema pallidum and bronchal spirochætosis. Louse-borne (or *Borellia Berbera*) relapsing fever was not endemic in the Sudan, but an epidemic introduced from Egypt was studied. The results of this last work were not published owing to the illness of the Director, but I have a vivid recollection of carrying the uninfected lice about in a metal container, kept in a warm pocket and feeding them on the forearm at regular intervals. It never occurred to us to feed lice contrary to their nature, that is to instil into their rectum blood from a fine drawn out glass pipette, as has since been done.

Vaccines were tested with varying success in " pyosis tropica," streptococcal puerperal fever, and cerebro-spinal meningitis. Material for the cerebro-spinal meningitis work was obtained from a small but very fatal epidemic; serum treatment was not available and there was not time to prepare an antiserum. The " trichonocardiases," a tropical hair infection, was investigated. " Sleeping sickness in the Lado of the Anglo-Egyptian Sudan " and " measurements of Dutton and Todd's Gambia strain of trypanosome " formed subjects for research and publication. Amœbic infections and their treatment with emetine were investigated.

As may be seen, a considerable proportion of the work was directed towards the investigation of human disease, but it was a matter for regret that no wards were attached to the laboratories.

To maintain a supply of material a limited amount of routine work was encouraged. The bacteriological examination of the Khartoum water supply formed one of the duties, and investigation of animals suspected of rabies was another. For the Pasteur treatment patients had at that time to be sent to the Public Health Laboratories in Cairo. Autogenous vaccines were prepared as a routine for treatment, and were standardised by Wright's red corpuscle comparison method or by counting with the dark-ground illumination. Microscopical Widal reactions

were still in use, but in the hot dry climate evaporation, even when the tests were kept in a moist chamber, tended to make them unsatisfactory. Dust was a great bugbear and plates became rapidly contaminated in spite of great care. When sand storms blew no man could work.

The Wassermann reaction had been introduced, but there was great difficulty in keeping guinea pigs alive. They fed ravenously, but as their excretion could not keep pace with their intake, the result was fatal. When their food was regulated they died of flagellate dysentery. Fleming's reaction was used, but as the results were not satisfactory it was not adopted as a routine method.

At about this time the antimony treatment of schistosomiasis was introduced, and the intermediate mollusc hosts of the *schistosomum hæmatobium* was discovered in Egypt by Leiper in 1915. In 1916 Inada and his co-workers in Japan published their work in English and showed that infective jaundice was a spirochaetal disease.

A number of problems presented themselves for investigation. Some have no counterpart in this country, but there were others which form part of our own problems. Of these the investigation of puerperal fever is one of the most urgent. Only a little time ago, Dr. C. J. McSweeney before this Academy stressed the necessity for this line of inquiry in Dublin.

Epidemic cerebro-spinal meningitis is another subject for investigation. In this connection it is interesting to retrospect. The late Dr. A. J. Chalmers, my chief in Khartoum, while working at the disease infected his finger, and from the lesion grew an organism morphologically and culturally identical with the meningococcus. There is some analogy between this and the finding of a streptococcus by the Dicks in 1923 isolated from the finger of a scarlet fever patient, with which organism they experimentally produced scarlet fever. The analogy might perhaps be brought further in the possibility of establishing susceptibility or resistance to the meningococcus, as has already been done in the case of diphtheria and scarlet fever. The occurrence of mycetoma in the tropics reminds one that quite a number of cases of actinomycosis present themselves in Ireland every year, and offer a field for investigation.

A good many of the tropical diseases are more easily attacked by preventive methods, for instance, mosquito and fly control. How much have improved conditions in Ireland affected the health of the community? what diseases have died out? and why?

Why are endemic leprosy and rabies unknown in this country? While working at relapsing fever I noted that Sir Patrick Manson, in the fourth edition of his book, *Tropical Diseases*, states "that the disease occurs especially in Ireland." The large group of industrial diseases will offer a fruitful field for research here in Ireland in the near future.

Towards the end of 1915 I was sent on a tour of investigation

up the White Nile, about 900 miles south of Khartoum, to the rapids where the White Nile debouches from the great lakes. On my return to Khartoum, owing to war emergencies I was posted to the Laboratory at Port Said. The work in this laboratory was routine, much augmented by war conditions. There was no gas, and primus stoves were used. The work included the examination of rats for plague, the investigation of possible cholera epidemics, the bacteriological examination of the municipal water supply (which was exceptionally good), the newly constructed filter beds at Kantara, and the water storage tanks of ships.

From 1916 to 1919 my narrative shows an unavoidable blank. In 1919 I was faced with a new and rather confusing world, my re-entry into which was ushered in with what appeared to be a new disease, *la grippe espagnole*, or pandemic influenza. A short period at the Liverpool School of Tropical Medicine, followed by an advanced course of pathology at the Royal Army Medical College, Millbank, made up to some extent for my absence from scientific work. Notwithstanding the War, pathological knowledge had increased and new routine methods had come into use, notably amongst them being the Wassermann reaction and its various modifications, blood grouping for blood transfusion, the Weil-Felix-Wilson reaction, the typing of organisms, the use of killed bacterial suspensions and the Schick test. To these might be added the culture and identification of the gas gangrene group of organisms, streptococcal infection of wounds, the recognition of " carriers " in an extended number of diseases, Brown's opacity tubes for vaccine work, and the hydrogen-ion standardisation of media.

In 1921, I was posted to the Eastern Command Laboratory at Woolwich. Here the laboratory was situated in the hospital grounds. The staff consisted of a pathologist, two trained laboratory assistants, and a laboratory attendant. The laboratory did the pathological work of the hospital. The clinical staff of the hospital offered every facility for co-operation. The laboratory was also a large Wassermann centre. The Wassermann reaction yet maintains its place, the triumph of a fallacy, the explanation of which still remains to be solved and the technique simplified. The problem is an interesting one, but is not an easy one for research.

A small epidemic of influenza was investigated, but the duration was so short that material soon became scanty. The findings were on a par with those of other workers of the time. It was soon realised that research on influenza requires a considerable staff and particular facilities, and that material flows and ebbs too quickly to allow of continuous work. Much advance has been made since then, but the necessity for further research is urgent.

In 1922, the Laboratory at the Citadel, Cairo, was revisited. A whole time pathologist was now part of its complement. Considerable laboratory extension had taken place, there now were

four sub-laboratories, two in Egypt and two in Palestine, and these laboratories were self-contained. The Wassermann reaction was now part of the routine, and bacillary and to a lesser extent amœbic dysentery, together with the identification of typhoid carriers, formed a very large proportion of the work. Some investigation was made into the serological grouping of dysentery bacilli. Agglutinating sera had to be obtained from England. Double Flexner infections were not uncommon, and late lactose fermenters in stools and urine interfered with the even tenor of the work. In this connection there is a wide scope for research in Ireland on diarrhœas of bacterial and non-bacterial origin. This field has been explored to a considerable extent in other countries, particularly in lunatic asylums. (Incidentally, this branch of medical service appears to offer an almost open field for investigation.)

From 1923 to 1927, professorial duties at the Egyptian University interrupted my direct association with practical clinical pathology. These years, however, cannot be allowed to pass over without noting the growth of biochemistry, particularly in America, and the almost universal adoption of various simplified and accurate aids to diagnosis, prognosis and the regulation of treatment. Banting and Best's isolation of insulin in 1922 did much to stimulate biochemical research and routine. The Dick test was added to our knowledge, and the discovery of the liver treatment of pernicious anæmia gave enhanced importance to blood examinations and to the fractional test meal.

A brief reference to Egypt is not out of place here. Egypt and Ireland are two countries where trachoma is common. Schistosomiasis looms largely in Egyptian pathology. Exuberant polypoid growths of the intestine as the result of irritation, and the tendency of the bladder lesions when infected to become malignant recall to mind the problems of neoplasms. This problem, and particularly that of carcinoma, forms one of the most urgent problems of the day. The solution offers a prize beyond the dreams of scientific avarice. Everywhere work on cancer is being done on a colossal scale, but to attempt this research in Ireland with any hope of solution would entail almost the establishment of a special institute. With regard to disease of the liver and spleen there is still scope for study in this country as well as abroad. To ensure an adequate and continuous supply of such material in this country cases would have to be collected into one hospital. The investigation of liver function alone forms a complete subject for research. In diabetes we have a field with numerous subsidiary avenues leading up to it; here the reason for the failure of the " islets " is still a problem.

The years 1927 to 1929 were spent at the Eastern Command Laboratory, the Queen Alexandra Military Hospital, Millbank.

The Command Laboratory had been moved to Millbank; the Woolwich laboratory and laboratories at Shorncliffe and Colchester were self-contained sub-laboratories. The laboratory

was situated in the hospital. The proximity of the Royal Army Medical College for help and advice, together with the use of the library, were of inestimable advantage.

Here was first brought to my notice the advanced scientific attitude of the clinicians. I do not wish to imply that this had not existed before, but it was now markedly in evidence. Clinicians discussed cases with the pathologist in the laboratory, wards and post-mortem room. " Team-work and co-operation " was the order of the day.

As the hospital collected all the interesting cases from home and abroad for investigation and teaching purposes it provided a wide field for investigation. On one occasion an epidemic of diphtheria was investigated; Schick testing on a large scale was done, and the active immunisation of a number of adults with the then latest product, " toxoid-antitoxin mixture " (which procedure was regarded as possessing certain dangers), was carried out without ill effects.

Some interesting problems presented themselves. Amongst them was the appearance of tuberculosis in middle life. A number of individuals, men who were approaching middle life, in fact due shortly for retirement on pension, and who had previously been perfectly well, developed pulmonary tuberculosis. These individuals all originally came from Ireland. Has this a counterpart in civil life? and what is the explanation?

Sprue was examined with reference to ulcers of the mouth. Although much research has been done already, there is still scope for investigation into the flora of the mouth in health and disease. There is the need for a single plate medium which will identify streptococci from pneumococci.

In pernicious anæmia, we have to consider from the standpoint of the laboratory: the blood count, the measurement of the red blood corpuscles, the van den Bergh reaction, the histamine-stimulated fractional test meal, and the reticulocyte count; from the clinical side, the signs and symptoms and the exercise of clinical sense. A truly formidable array. Nevertheless we read in the books: " if the patient does not react to liver therapy, the disease is not pernicious anæmia." There still exists the necessity for some more definite means of laboratory diagnosis, particularly in borderline cases.

As a subject for post-mortem investigation, the cause of sudden deaths in young adults, which are not attributable to malicious or accidental circumstances, might form the subject of an interesting report.

From 1929 to the present, my time has been spent at the Mater Misericordiæ Hospital. As a lone pathologist to the largest hospital in Dublin, the pathological, bacteriological, and biochemical routine, and the teaching duties connected therewith, have cast their mantle on me, a not unworthy mantle worn by such famous predecessors as the late Professor E. J. McWeeney and Professor W. D. O'Kelly. The enthusiasm and driving force

of my clinical colleagues at the Mater has been exhibited for many years before this Academy.

A new and spacious laboratory is now in the process of erection.

There arise in the routine work a number of constantly recurring difficulties and problems which call for research. Thus, there are constantly arising a number of blood pictures which are far from normal and are as yet of unexplained origin. Themes which suggest themselves as subjects for research include bone marrow hæmatopoiesis and improved methods for the selective staining of embryonic or immature cells in the bone marrow and blood; accurate hæmoglobin estimation; the hæmoglobin content variations with age, sex and surroundings; diseases of the white cells of the blood and, should opportunity present, agranulocytic angina.

Arising out of investigations of the digestive tract, we might seek the possibility of some better test meal substance than gruel; we might investigate the normal and abnormal bacterial flora of the stomach and their effects, if any, on ulceration of the stomach and duodenum.

Urinary problems which crop up daily include a further survey of the Conway test for renal function and its prognostic significance, and the coliform infections of the urinary tract.

Amongst general problems, toxic goitre has gone beyond our present knowledge of histological changes. A survey of toxic goitre in the Irish Free State is long overdue. Heart disease in young adults, and acute and chronic rheumatism, form fruitful fields.

In the tuberculosis field, we might enquire into the reason why only some members of a family in the same house develop pulmonary tuberculosis, as into the geographical distribution of the human and bovine types of tubercle bacilli in the Irish Free State.

In helminthology, a review might be made of the cestode infestations of man in the Irish Free State, with special reference to diphilobothrium infection.

Reports by clinicians on the efficacy of vaccines show that while there is no doubt that autogenous vaccine treatment in a number of cases is strikingly successful, in other cases apparently similar, no benefit is obtained. Does this depend on the composition of the vaccine or the reaction of the patient or on both? Autogenous vaccines and vaccine therapy require further investigation, particularly as regards the type of organism used in prophylaxis. In what form will a bacillus evoke the maximum protective response?

This brings me to the end of my Odyssey. From it, I think it may be seen that even within the limited experience of one individual there arise many problems calling for further investigation. I do not by any means suggest that the list which I have given

is complete, but the subjects mentioned have all come more or less within the ambit of my work.

Needless to say, before embarking on a particular line of research there is a large field of preliminary investigation to be explored. The literature of the subject chosen, as it has appeared in the various journals and research reports, requires to be thoroughly studied, so that one may be cognisant of, and fully appreciate, what work has already been done, what work is being done, what new conceptions put forward, and what new conclusions arrived at

I have been constrained to tell the tale as I have told it because I believe there are also certain inferences, with regard to facilities for research, to be drawn from it. As a result of my experience I venture to set down in more or less tabular form the points which I consider essential for any form of clinical laboratory research.

(A). *Primary essential:*—There must be sufficient laboratory accommodation.

(B). *The Worker:*—The worker should be adequately paid. He must have some practical experience, be keen on his subject, and devote himself so far as possible to a single problem.

(C). *Facilities:*—The worker should be allowed ample time and library facilities to enable him to study his subject. Adequate equipment and a sufficient number of technical assistants should be provided. The laboratory must be in the hospital, and full access should be given to the wards and records. He must be free from interference and extraneous routine work. He must have the willingly given assistance and co-operation of the clinical staff, who should not stand *in loco parentis*. The worker should be at no personal expense in publishing his work.

Finally: Satisfactory clinical research can be best conducted by a team consisting of a pathologist, a bacteriologist, a biochemist and such other experts as may be found necessary, all working in collaboration with the clinician, and the whole team working as a unit,

ONE FOR ALL AND ALL FOR ONE—HUMANITY.