THE WEIL-FELIX TEST FOR THE RICKETTSIOSES

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Prologue

A pathologist's lot is a happy one. Some things he does are to say the least, most extraordinary even unbelievable, except that they are practical and therefore useful. Consider the bullock's heart, freed from fat and alcohol extracted — now use the resultant product to diagnose Syphilis in Switzerland, Pinta in Paraguay or Yaws in Zanzibar. Go down to the local abattoir and collect the dripping blood from a bleeding sheep, or better still take some horse's red blood cells — now mix them with the fluid portion of your jaundiced patient's blood and you may pontificate that he or she is suffering from Infectious Mononucleosis (or should it be Infectious Mononucleoses?)

And this goes on and on; some months ago a patient fresh (I speak metaphorically with reference to an episode in time and not to the patient's actual physical condition at that time — perhaps "fresh" is not the right word) from his Mediterranean holiday was complaining of a P.U.O. (or if you are an American colleague, an F.U.O.). An aliquot portion of a specimen of his serum was therefore prudently referred for investigation re leishmaniasis. The pathologist in his ivory tower screened the patient's serum against this protozoal disease by testing the specimen against — of all things — B.C.G. used as a diagnostic reagent in his laboratory test!

Now take this Weil-Felix test; the other day a young Caucasian patient coming out of Africa developed fever with a rash soon after landing in his native Britannia. One of the possibilities was obviously a rickettsial fever — so off went his serum for screening against rickettsial disease, and one of the tests done was, in fact, the Weil-Felix test, that is to say doubling-up dilutions of the patient's serum from 1 in 20 to 1 in 10,240 are placed in a row of test tubes and a drop of a milky suspension of Proteus bacilli is pipetted into each. The test-tube rack is then placed in an incubator at 37°C for 2 hours, stored overnight in the cold room, and the results are observed after a further 2 hours standing on the laboratory bench at 22° C. After this cabalistic procedure the pathologist duly reports his findings.

It was at this point that I sat up (so to speak) and took upon myself the task of finding out the How and Why and Wherefore of this Proteus bacillary suspension test to screen for such an exotic illness as a spotted-fever-group communic-

able disease.

With these objectives in mind I betook myself to those Herculean Pillars of British Medicine, "The Lancet" and the "British Medical Journal" optimistically expecting to get an early answer to the mystery of the W-F and thereby achieve a quick Q.E.D. and consequent peace of mind. This however was not to be; the Quest for the W-F was not to prove so easy.

Section 1. Stadium 1

The Quest begins . . .

To set down in correct chronological order the sequence of events as the story unfolds itself, the first finding that the search threw up was a very inconspicuous note right at the bottom of a page in the "British Medical Journal" for May 19th, 1917, page 649, where it was announced for the diligent reader who reads medical journals from front page to back page (including the adverts) that,

> "The German authorities have arranged for the systematic testing at the Robert Koch Institute of blood taken from patients suspected of typhus fever. The Weil-Felix reaction is employed, and whenever the reaction is negative but the clinical symptoms remain suspicious a second sample is examined".

This was indeed only a meagre 5-line note but it was certainly calculated to arouse the curiosity of an amateur medical historian such as I am, especially taking into consideration the fact of my being also an army man at that. The reader will note the year — this was in fact in the midst of World War I, and I thought to myself as I read this notice that once more our German medical confreres had got in first again — a là Wassermann and his evergreen venereologic test.

This was then the beginning of the trail, that kept on opening hitherto unsuspected vistas to me, as I coasted along it; very much like those exhilarating and wonderful mountain roads in the Tirol. For no sooner had I started along the main highway, when my observation was drawn to the fact that possibly I had not began at the very beginning. But let each reader judge for himself.

On June 16th, 1917, a month later that is, the "British Medical Journal" on page 825, carried an anguished *cri du coeur* in protest against the previous innocuous-looking and half-hidden announcement. Here are extracts (somewhat paraphrased) of these, which were in the form of a letter entitled,

"Typhus Fever and the so-called Weil-Felix Reaction"

note the emphasis on the operative words "so-called".

The letter read as follows,

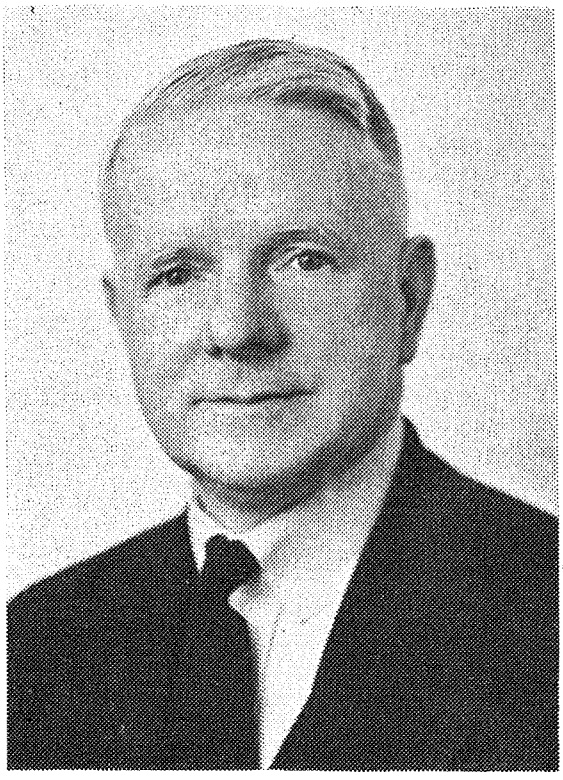
"Sir,

I read this with great interest, since it shows that investigations carried out by me at Belfast some ten years ago are now being confirmed on an extensive scale. The results of my investigations were published in the "Journal of Hygiene" (the writer refers to J. Hyg. for 1909, and 1910). In these publications I showed that in typhus fever the blood serum often agglutinates intestinal bacilli — for example *B. coli, B. typhosus,* and especially a coliform bacillus isolated from the urine of certain cases. I pointed out in the paper dealing with the etiology of typhus fever that the presence of these agglutinins did not necessarily imply that the bacillus in question was of etiological significance. The whole question of heterologous agglutinins is fully discussed in my paper, and before the reaction is credited to Weil-Felix, I think it but fair that my work on the subject should be considered.

It would seem therefore that the work of Weil, Felix and Dietrich (Deutsch. med. Woch. 1916) is a rediscovery of what was pointed out by me in the "Journal of Hygiene" many years ago.

Signed:

Captain W. James Wilson RAMC (TF)
No 54 (London) General Hospital
British Expeditionary Forces,
May 25th.



WILLIAM JAMES WILSON (1879-1954)

"I showed that in typhus fever the blood serum agglutinates a coliform bacillus isolated from the urine. The work of Weil and Felix is a rediscovery of what was pointed out by me many years ago."

William James Wilson (1879-1954) had been Emeritus Professor of Public Heath, Queen's University, Belfast and Consultant Director of Public Health Laboratory Service, Northern Ireland. He was also noted for the Wilson-Blair medium used especially for isolation of *Salmonella typhi*; on this medium the first positive isolations of this dangerous organism from sea water and from River Thames water had been reported.

On our side of the barbed-wire fences some of his fellow war service medical officers gave Capt. Wilson a measure of loyal support; thus late in 1918 (Sept 21) "The Lancet" published an article entitled "Typhus Fever" by Capt. C.M. Craig, Royal Army Medical Corps, and Major N. Hamilton Fairley, Australian Army Medical Corps. These two authors subtitled their paper, 'Observations of a serological test (Weil-Felix Reaction)' but nevertheless acknowledged Wilson's work in their very first introductory paragraph; thus they opened up their paper by stating:

"The following is a brief account of our experience of the Weil-Felix reaction during the early months of 1918 in Egypt and Palestine. The reaction is an amplification of the work done by Dr. Wilson of Belfast who first showed that typhus serum possessed the power of agglutinating certain intestinal organisms. During the past year it has been favourably reported on by Austrian and German workers."

Sir Neil Hamilton-Fairley (1891-1966) was one of the most distinguished Australians of his generation and an outstanding worker in the field of tropical medicine.

EDMUND WEIL (1879-1922)

"The Weil-Felix Reaction was one of the most outstanding medical achievements of World War I, and it was indeed suggested that to honour Weil the Proteus X strains should be called Proteus weilii".

He described a new pigment in blood, methaemalbumin, and was elected F.R.S. in 1942. His work as Director of the Land Headquarters Medical Research Unit, in World War II in Queensland, Australia led to the Allied Armies prophylactic anti-malarial regime of daily mepacrine. He was the first holder of the Wellcome Chair of Tropical Medicine at London University in 1946 and a Consultant to the British Army in Tropical Medicine in 1950.

In expressing their acknowledgement Capt. Craig and Major Hamilton-Fairley were in fact reiterating the sentiments expressed in an editorial annotation which appeared in the British Medical Journal for Jan. 12, 1918. I found this annotation most intriguing and I like to imagine that it must have been doubly more so to the service medical officers of our armies who were thus kept informed of the medical advances on the other side of the trenches. Selected paragraphs are quoted below; I hope that judicious but necessary pruning on my part does not detract from the intrinsic fascination which this annotation will hold for the reader of today, and which indubitably it must have held for our forebears then fighting in the various theatres of World War I.

ARTHUR FELIX (1887-1956)

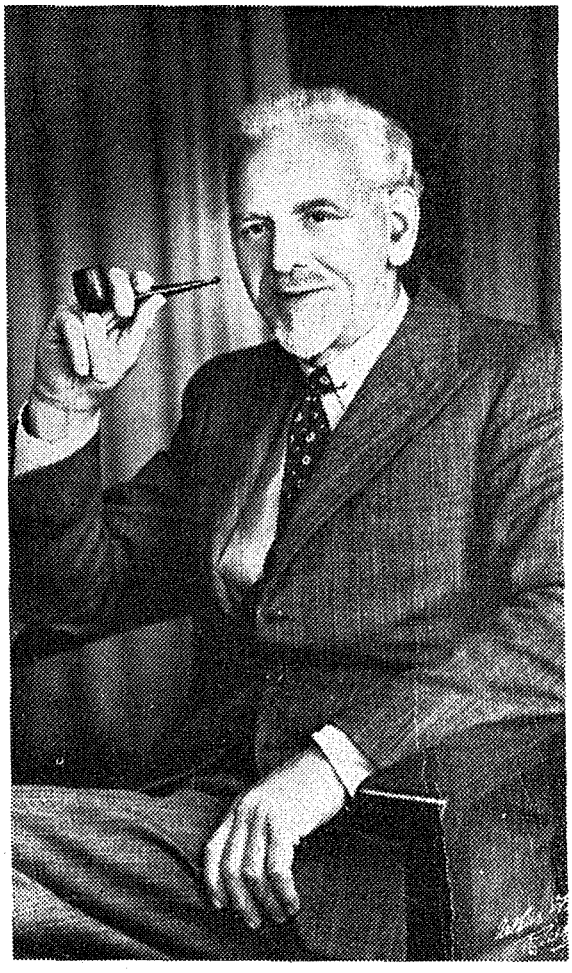
"Three features of the agglutination reaction with B. proteus X19 led to its rapid recognition as the decisive criterion in the diagnosis of typhus fever."

THE WAR

Typhus fever: Laboratory Diagnosis

The so-called Weil-Felix reaction as a method of diagnosis in typhus fever has been widely accepted by German workers, especially on the Eastern fronts, where in the Balkans especially, cases of typhus fever have been numerous. The earliest observations on the subject would appear to have been made by Dr. W. James Wilson in Belfast. He published two papers on the subject in the "Journal of Hygiene" in 1909 and 1910 respectively. He showed that in typhus fever the blood serum often agglutinates intestinal bacilli — for example *B. coli*, *B. typhosus*, and especially a coliform bacillus isolated from the urine of certain cases. As the reaction now known in Germany by the name of 'Weil-Felix' would appear to be applied on a large scale it seems well to give some account of the original communication of these authors.

Weil and Felix (Wien. med. Woch, 1916) were investigating a group of cases in Poland thought to be enteric fever, but in whom the Widal reaction against typhoid, paratyphoid A and paratyphoid B was negative. One of the cases was a Roumanian doctor; and from his urine an organism was isolated which was not only agglutinated by this patient's own serum (at a dilution of 1 in 200) but also by the sera of nine other patients. This organism was a short motile Gram-negative bacillus with the characteristics of *B. proteus*. By vaccinating a rabbit with two doses of the bacillus, a diagnostic serum was obtained with a good working titre of 1 in 2000.



ALAN NEAVE KINGSBURY (1889-1965)

"Dr. Kingsbury's strain was the only one to give a specific Weil-Felix reaction when tested against tropical typhus patients' sera."

Some time later Weil and Felix, now working in Russia, again found that sera from typhus patients agglutinated their proteus-like organism to a titre ranging from 1 in 100 to 1 in 200, while numerous specimens of sera from patients suffering from diverse diseases such as typhoid, dysentery, pneumonia, bronchitis, pleurisy all either gave negative results, or a positive reaction only up to the very low titre of 1 in 25.

'Weil and Felix continuing their research on this problem succeeded in isolating yet another serotype of the same bacillus, and to this strain the name X19 has been applied, the first strain being called X2. The essential difference between the two strains is that X19 is agglutinated up to a much higher titre by the serum of typhus cases, up to a dilution of 1 in 2000, or even higher. Thus the reaction with this strain is much easier to work with than the much less agglutinable original strain X2.

Much discussion has circled round the problem of whether this organism is to be considered in any way as the cause of typhus fever. The general consensus of opinion is that it is not, and this is the belief of Weil and Felix themselves. The most generally accepted opinion is that the organism is a specific secondary invader of the body which always accompanies the unknown virus of typhus fever".

World War I was responsible for some o f the worst epidemics of typhus that the world has known. The notorious Serbian epidemic of 1915 was one of the greatest in extent and most sudden in onset in history; in 1917-1918 there was a tremendous epidemic in Roumania. From 1916 to 1919 there were over 400,000 cases in Poland and Galicia. The figures for Russia were horrific; between 1917 and 1923 there were 30 million cases, with a mortality of 10% in European Russia.

In this day and age everyone accepts unquestioningly the fact that the causative agent of epidemic typhus is *Rickettsia prowazeki* but in those days the candidate etiological agents were as numerous as candidate viruses have been in our days for the virus of infective hepatitis.

Rickettsia Prowazeki was the name given by the Brazilian worker da Rocha Lima to commemorate the names of those two great investigators, Ricketts who first associated organisms of this genus with spotted fever and typhus, and van Prowazek, another early investigator of the etiology of typhus. Both these pioneers, at the early age of 39, fell victims to the dreaded typhus agent. The American Howard Taylor Ricketts died in 1910 in Mexico while investigating Tabardillo or Mexican Typhus, and the Austrian Stanislaus Joseph Matthias van

Prowazek, (who had also in 1907 in Java described the characteristic intracytoplasmic inclusion bodies in conjunctival smears from trachoma patients known as Halberstaedter-Prowazek bodies) succumbed while investigating typhus in Serbia in 1913. Henrique da Rocha Lima (1879-1956) is himself commemorated in the genus Rochalima, whose members differ from the genus Rickettsia in that these organisms can be cultivated on cell-free media e.g. *Rochalima quintana*, the etiological agent of another well known war disease: Trench Fever. Da Rocha Lima worked in association with Prowazek, and also was infected with typhus but fortunately survived.

In the early part of World War I W.W.C. Topley, in the Royal Army Medical Corps wrote up a series of 30 cases of typhus which were investigated in his laboratory attached to the British Military Sanitary Expedition to the Serbian Forces — see Journal RAMC, 1915. In his discussion Captain Topley surveys some of these candidate etiological agents, and comments inter alia substantially as follows:

"Since the method of obtaining cultures from considerable quantities of blood drawn from a large vein has come into vogue, a considerable number of investigators have isolated from typhus cases organisms of coccal or cocco-bacillary forms: Wilson in 1910 obtained Gram-positive bacilli in fifteen out of thirty-three cases of typhus fever. In one case he obtained a Gram-positive diplobacillus; by means of *agglutination reactions* he demonstrated the presence of specific antibodies in the blood of typhus patients. Rabinowitsch in 1909 during an epidemic in Kreff isolated a short bacillus with rounded ends; he demonstrated a certain degree of *agglutinating power* against these organisms in the blood serum of typhus patients, though the results do not seem to have been constant. Predtjetschensky in 1910 during an epidemic in Moscow isolated from every case a short Gram-negative bacillus showing polar staining, but *agglutinating reactions* gave no certain result. Fuerth in 1911 in Tzingtan isolated a short bacillus which was Gram-positive on first isolation but tended to become Gram-negative on cultivation. *Agglutination reactions* were inconclusive.

Hort and Ingram in 1914 examined the blood in 22 cases of typhus occurring in Ireland and obtained without difficulty cultures of diplococcal and diplobacillary organisms. A similar coccobacillary organism was also found in the C.S.F. in one case.

It must be noted that a large number of investigators have failed to obtain any growth in cultures obtained from typhus blood."

William Whiteman Carlton Topley (1886-1944) became Professor of Bacteriology at Manchester University and later Professor of Bacteriology and Immunology at London University. He was elected FRS in 1930 and helped to set up the Emergency Public Health Laboratory Service in World War II. Member of the War Cabinet Scientific Advisory Committee in World War II. Famous co-author, with Wilson, of Topley and Wilson's Principles of Bacteriology and Immunology which remains even today an international reference book in its field.

The reader will note that generally speaking these investigators failed to get conclusive results in specific agglutination tests using their candidate micro-organism; and besides those in the list given above there were numerous others. Thus Wilson and Darling rushed rather hurriedly into print (Lancet, Apr. 14, 1917) to describe an organism they thought they had found, using dark-ground microscopy of the blood,

> "in our opinion the micro-organism is closely related to *Spirochaeta icterohaemorrhagica* and to the bacillus found by Wolbach in Rocky Mountain Fever, and pending further investigation might be described as the *Spirochaeta typhi*."

Alas, this organism was nothing else but an artefact, and Wilson withdrew his claim as hurriedly as he had made it. Similarly, fresh from their discovery of the rat-bite fever spirillum, Japanese workers also reported a spirochaete in the urine of patients, for which they proposed the name *S. exanthemata typhi*.

Simeon Burt Wolbach (1880-1954) was

a famous teacher at Harvard, being Shattuck Professor of Pathological Anatomy, 1922. He was known for his work on typhus — in 1919 he described *Dermacentroxenus rickettsi* as causal agent of Rocky Mountain Spotted Fever, and demonstrated its intra-nuclear life cycle in tick tissue. In 1920 headed the Red Cross Societies expedition to Poland to discover the cause of an epidemic of typhus. Consultant to the Walter Reed Armed Forces Institute of Pathology, he was a joint author with Todd and Palfrey of the famous textbook 'The Etiology and Pathology of Typhus'.

Another organism whose claims had to be considered more seriously (judging from my review of literature of that time) was the anaerobic Gram-positive Plotz-Olitsky-Baehr bacillus, and named *Bacillus typhi-exanthematici* at the suggestion of Professor W.H.H. Welch, the name having been originally suggested by Klebs for the hypothetical infective agent of the disease (Lancet, 1915). This organism was stated to react quite specifically in complement fixation tests, agglutination tests and precipitation tests with patients' sera (J. Inf. Dis., 1915); and a vaccine was even prepared from it for the immuno-prophylaxis of typhus. Its sponsors not only described subclinical typhus infections diagnosed serologically by detecting antibodies to their bacillus (Baehr — J. Infect. Dis. 1917) but even enthusiastically enough removed lice from typhus patients and recorded that 'from practically all these lice the *Bacillus typhi-exanthematici* was isolated sometimes in pure culture." (Olitsky J. Amer. Med. Ass. 1917.)

On the other hand Weil and Felix (Z.I. Immunitatsf. 1921) went to the other extreme and suggested that the non-culturable parasitic typhus virus is (exceptionally and under unknown conditions) transformed into the culturable saprophytic stage known as *Proteus X*. One of the facts on which this hypothesis of the transformation of typhus virus into *Proteus X* is based is the occurrence of X strains in close connection with the disease. All the genuine strains of *Proteus X* strains which were tested by Weil and Felix (Wien. Klin. Woch., 1918) had been isolated by these workers and others from cases of typhus in various parts of Europe and Asia Minor. Twenty-four of these strains were classed as type X2 and thirty-one strains as X19. On the other hand, out of 126 cultures of *Proteus vulgaris* including nearly all cultures which in 1918 existed in any collection in Austria and Germany, none was found to possess the main antigen of type OX19 or OX2.

In 1956, G.S. Wilson (1895-), professor of Bacteriology at the London School of Hygiene and co-author of the famous text-book which has become the Bible of British Bacteriologists, wrote in the "British Medical Journal" that "the demonstration in the early nineteen-thirties by Bruce-White in England and by Castañeda in America of an antigenic factor common to these organisms serves to explain the rationale of the W-F reaction".

However that may be of all the tests and claims mentioned above the Weil-Felix Reaction was the one that stood the test of time. As Felix stated many years later (1927 — Med. Res. Council System of Bact.):

"Three features of the agglutination reaction with B. proteus X19 led to its rapid recognition as the decisive criterion in the diagnosis of typhus fever:—

(i) The almost absolute constancy of the phenomenon with the serum of typhus patients. (ii) The high titre of agglutination attained in the great majority of cases. (iii) The rarity and low titre of 'normal' agglutinin for X19.

In everyone of these respects — essential as they all are in determining the value of a diagnostic test — the position of the X19 reaction is superior to that of the classical sero-diagnostic method, the Widal test in typhoid fever".

And in 1944 Felix (now working in England) drew attention to the various modifications of the original W-F test as were then being adapted and adopted by the German war time medical services in World War II (see Trans. Roy. Soc. Med. Hyg., 1943-44).

"German workers have also been employing slide-agglutination tests since

the beginning of the present war (World War II), and have published a great number of reports describing various modifications of the technique. Preserved suspensions of Proteus OX19 are distributed from central laboratories and the test is carried out by mixing a drop of finger blood, or of the separated serum with a drop of the concentrated suspension. Some of the German military laboratories issue the OX19 antigen in the form of an alcoholised or formalised suspension, stained with methylene blue; others send out slides on which a number of drops of the concentrated suspension have been dried. Dried cultures of Proteus X19, reduced to a fine powder, are also employed. Another procedure is to collect the specimens on glass slides in the form of dry smears of whole blood and test subsequently by adding a drop of the antigen. The tests are read with the naked eye according to the intensity and rapidity of clumping and it is stated that the results compare favourably with those obtained with test tube agglutination. The slide tests are employed in epidemiological surveys of large communities and mild cases and inapparent infections may be detected by this means. The test is also used in rapid bedside diagnosis in field conditions. Some of the German workers accept the results of slide agglutination as final, while others employ the test as a preliminary to the customary tube test".

In the early days of the W-F reaction (Munch. med. Woch., 1918) Weil and Felix insisted on using freshly prepared suspensions of live organisms; while in the expert hands of these two enthusiastic and meticulous workers this procedure proved most reliable, it was far from being convenient or even practical for the ordinary laboratory pathologist or bacteriologist. Hence we find Z. Bien and F. Sonntag (Haltbaren Fleckfieber-diagnostikums-Munch. med. Woch. 1917) putting forward the advantages of using alcohol-treated suspensions. A good account of alcohol-prepared and formalin-preserved suspensions that I came across is that given by Lieutenant-Colonel R.F. Bridges in the Journal of the RAMC, 1935. Other early workers proposed the use of heat which appeared to neutralise the 'H' non-specific antigen common to all proteus bacilli, leaving intact the more heat- stable 'O' receptor specific to the Proteus X strains; yet another method suggested was to kill cultures of X19 by the addition of 1 per cent solution of phenol, or to grow Proteus X strains on carbolic-acid agar where again the 'H' antigen was suppressed. (H. Sachs — Deutsch. Med. Woch, 1917 and 1918; H. Braun — Berl. Klin. Wschr., 1918: Karl Csepai's Dauerdiagnostikum — Wien. Klin. Woch, 1917 and Munch. med. Woch, 1919; Bruno Neuber — Munch. med. Woch, 1917).

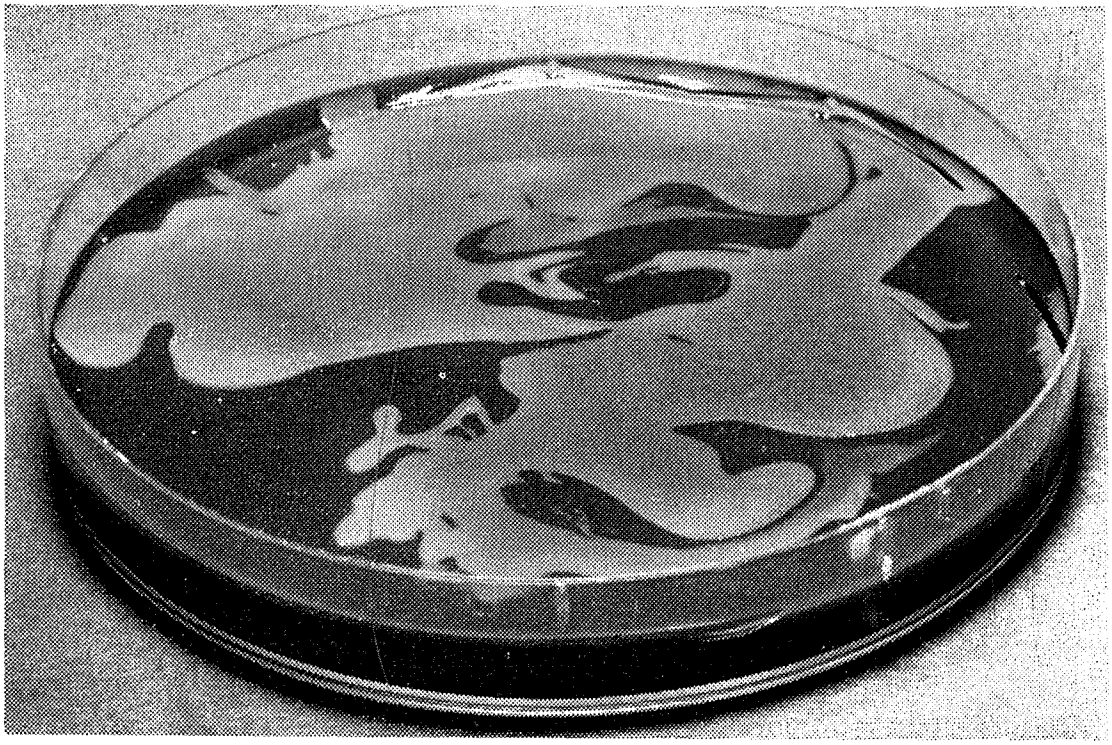
The Proteus O and H forms

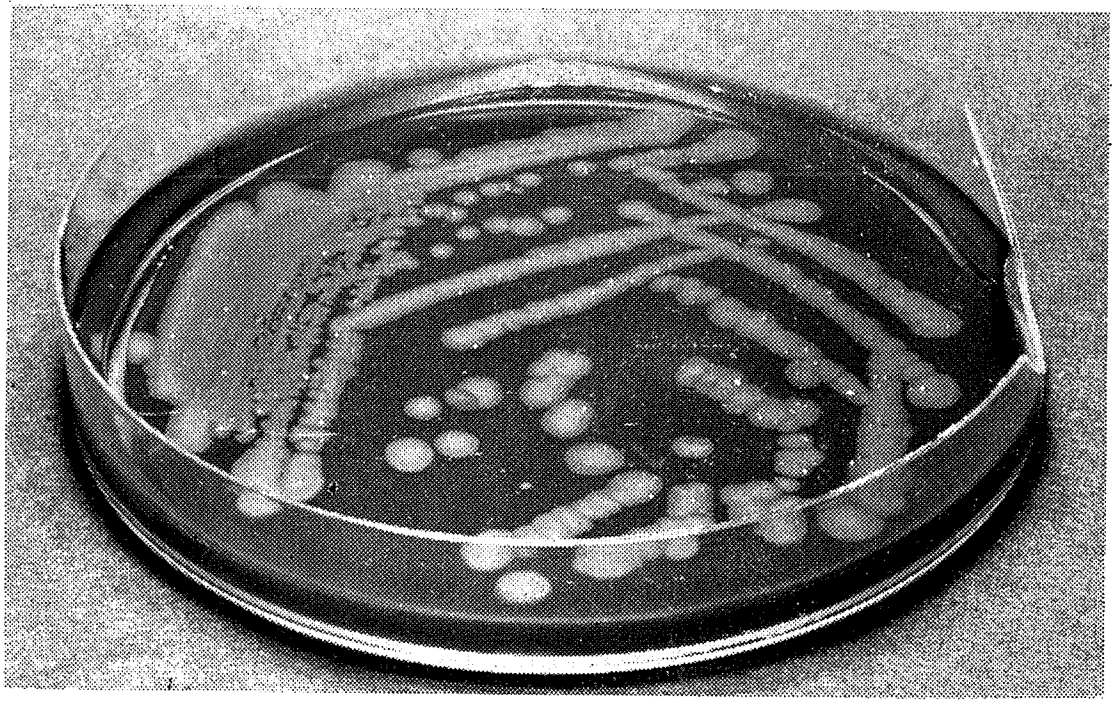
Weil and Felix (Wien. Klin. Woch, 1917) found that while the serum of immunised animals agglutinated all forms of Proteus bacilli, that from typhus patients had a narrower spectrum and acted only upon their special series of Proteus X strains; they observed (from an old culture of X19) that X strains exhibit on artificial culture medium 2 distinct forms which differ from each other morphologically, biologically and serologically.

Firstly, there is the filmy spreading growth characteristic of a Proteus bacillus. I often demonstrate this to my students by breathing on a pane of glass. To this form Weil + Felix gave the name 'H' colonies from the German *'Hauchformig'*. Cassell's New German Dictionary defines *"Hauch"* as breath, exhalation, haze, bloom, aspiration.

Secondly, the bacillus can grow in small, discrete rounded colonies, which Weil and Felix called the 'O' form i.e. *Ohne Hauch* — without a film.

Serologically the 'O' forms were considered to possess only one kind of receptor which was, however, specific for typhus fever. In agglutination tests the 'O' bacilli precipitated in fine firm granules the reaction proceeding slowly; the 'H' form bacilli were more complex and were considered to have, in addition to the above typhus specific receptor, another





"They observed that X strains exhibit on artificial culture medium 2 distinct forms: a filmsy spread growth (H forms) or discrete rounded colonies (O forms)". Shown here in Fig. 1 and Fig. 2 are these characteristic appearances of Proteus HXK (NCTC 7827) and Proteus OXK (NCTC 8309) deposited in the National Collection of Type Cultures (British) by Felix in 1949-1950.

receptor common to all Proteus bacilli. The 'H' bacilli reacted more quickly in agglutination tests and produced large heavy coarse floccules.

This account reminded me very much of *Vibrio cholerae,* upon which I was working at the time of writing this essay, where one finds specific 'O' antigens for the *Vibrio cholerae* serotypes, and 'H' antigens which are common to vibrios of all groups and hence not of much use in serological diagnosis. I noted also that the opposite holds good in the salmonella group of bacilli where the specific antigens are in fact the 'H' phase 1 antigens.

The importance of the 'O' and 'H' forms recorded by Weil and Felix had a greater and more far reaching consequence above and beyond its immediate intrinsic interest as appertaining to the W-F reaction proper — but that is another story, and to continue with my present line of thought, it would appear that Weil and Felix missed the point that the characteristically spreading 'H' forms of proteus bacilli correlated with the motile forms and the 'H' antigen correlated with possession of flagellae. It was left to Braun and Schaeffer (Z. Hyg. Infektkr., 1919) to record this, while as far back as 1903 the American workers Theobald Smith and A.L. Reagh (J. Med. Res.) in a paper read at the 3rd Annual Meeting of the American Association of Pathologists and Bacteriologists stated that the "agglutinin acting upon the bodies of the non-motile hog-cholera bacillus (*Salmonella cholera-suis*) is identical with that acting upon the bodies of the motile race or species, but different from that acting upon the flagella". These flagellar and somatic antigens, as the reader will now observe, correspond to the Weil and Felix 'H' and 'O' forms respectively.

On an exciting day in 1927, a worker (Sir Joseph Arkwright 1864-1944) at the Lister Institute of Preventive Medicine, London watching under his microscope with bated breath the agglutination of living motile organisms observed that while 'H' agglutinins threw the bacilli into immobile groups, rather loosely knit, the 'O' serum caused the bacilli to clump but these groups continued to tour the microscopic field of vision.

Section 1. Stadium 2

EDMUND WEIL and ARTHUR FELIX

Leaving aside for a brief interlude the strictly scientific quest, a detour in my search was purposely undertaken to discover more of Weil and Felix as persons and individuals rather than as a double-barrelled eponym for a test, no matter how valuable for diagnosis and how prominent in history the W-F reaction has become.

It appears that Arthur Felix (1887-1956) who had successfully obtained a research doctorate in organic chemistry at the University of Vienna (actually for a thesis on indigoid dyes — no doubt very practical and useful for his family business as his father Theodor Felix had a commercial interest in printed textiles), was eventually caught up in the war upheaval and was called up for military service during the First World War. As at that time he was working in a microbiology laboratory (a mycology laboratory directed by Lafar in Vienna) he asked to be assigned to a similar laboratory and was duly drafted to a field medical unit on whose staff happened to be Oscar Bail, Professor of Bacteriology, and his Assistant Professor Edmund Weil, both from the Institute of Hygiene, Prague. Professor Bail soon left this unit to return to his former post in Prague, while Weil and Felix started a close collaboration which was to bring them both international acclamation, Weil being at this time for obvious reasons the more experienced of the two on the subject of bacteriology and serology.

It is not likely that A. Felix could be mistaken for anyone else. I wish I could state in this paper that I had met this great man, but the only thing Felix and I had in common was a habit of using red, blue, black and green coloured inks in recording experimental data: in work done at the British Army (The David Bruce) Vaccine Laboratories, East Ever-

leigh, England, following a tradition which was insisted upon and adhered to by my then Commanding Officer Colonel M.H.P. Sayers O.B.E. later Major-General M.H.P. Sayers, Director of Army Pathology at the War Office and at present Consultant Pathologist, Medical Services Division, Department of Employment, himself a pupil and a great admirer of Felix, who also taught me in turn the finer points of Felix's techniques of agglutination procedures.

Weil (1880-1922): Students sometimes ask whether Weil of the Weil-Felix reaction is the same person as the one associated with Weil's disease; I therefore hasten to say that the answer is in the negative as the latter, Adolf Weil (1848-1916 from Wiesbaden, Germany) was the Professor of Medicine at Heidelberg University who in 1886 described four patients (a chemist, a soldier, a merchant, and a curate) as suffering from a new form of infective jaundice, though he could not at that time demonstrate the infective agent — later shown to be the *Leptospira icterohaemorrhagiae*. And, as a matter of interest to clinicians, there was even another Edmond Weill (1858-1924 from Lyons, France) who gave his name to a sign in clinical diagnosis (Weill's sign) denoting the diminished movement of the subclavicular region of the chest in Acute Lobar Pneumonia.

At the time, when these two workers joined forces typhus was rife all over the Eastern Front as I have already indicated, and on September 15, 1915 when Weil and Felix were on the posted strength of No. 5 Austrian Epidemiological Laboratory (sited in Wadowice a town 25 miles to the south-west of Cracow and not far from Felix's own birth-place of Andrychow) they isolated, to some extent accidentally, the first of their famous series of organisms — a non-motile organism, which in fact was not immediately identified. Indeed Weil and Felix at first thought they had isolated the causal organism of a typhus-like/typhoid-like disease then rampant in the neighbourhood, and which was consequently referred to as X1. This organism was packed away for a few days while the laboratory was on the move through the exigencies of military service, and a fortnight later it was fortunately found to have assumed a motile form.

It was therefore revealed in its true colours and duly recognised and identified as a member of the Proteus group, and accordingly it was then called Proteus X1.

The name Proteus was first used in bacteriological nomenclature by Hauser in 1885 — it signifies changeability of form as personified in the Homeric poem by Proteus 'the old man of the sea' who had the gift of endless transformation. Hauser described a group of organisms which he had isolated from putrefying meat and classified them under 3 groups — *P. vulgaris, P. mirabilis, P. Zenkeri.*

Weil and Felix were firmly convinced, early in their investigations, that the agglutination of their X strains was specific and indicative of typhus, and it appeared to have become quite a popular test, one fancied by their fellow bacteriologists in near-by located field hospital laboratories, who indeed started using it with success for the diagnosis of suspected typhus patients. Nevertheless some doubt must have been inevitably expressed by some Austrian service medical officers who accordingly challenged Weil and Felix by handing over a batch of sera from patients suffering from assorted diseases. Felix spent Christmas of 1915 at the laboratory bench sorting out these sera while his brother officers were enjoying their 'Weihnachten' seasonal festivities. The results reported by the W-F reaction were fully substantiated, and the test was thereafter accepted as a useful laboratory aid in the diagnosis of typhus. Weil and Felix's paper described this historic happening in the Eastern Front was published in 1916 — I assume that as a matter of courtesy and correct medical etiquette, and probably through genuine professional respect, Weil sent a copy of the paper and a culture of their bacterial X strain to his Chief, Professor Bail in Prague because we know the latter tried out the test himself, agreed with and confirmed their observations and added a

post-script to their paper 'Zur serologische Diagnose de Fleckfiebers', published in the Wien. Klin. Woch. for January 13th, 1916. In his note Professor Bail referred to this test for the first time in medical literature as the "Weil-Felix Reaktion."

Inter alia it is sad to record, (but life's like that), that the Director of the Austrian Army Medical Service who may have disliked Jews, in an effort to put the brakes on Weil and Felix's investigations ordered the re-siting of their laboratory further east nearer the Russian Front, and later on posted Weil to Durazzo in Albania on the pretext that there was an outbreak of cholera in Albania and an experienced bacteriologist was needed. Felix meanwhile anxious not to stop working at their previous Weil-Felix reaction succeeded in obtaining a letter of recommendation from Wassermann in Berlin (who incidentally was not on particularly good terms with Weil, as the latter had expressed some doubts on the specificity of the Wassermann reaction) to some influential official in Constantinople. Here Felix worked in German Red Cross Hospital and once more he had opportunities to substantiate the diagnostic value of the Weil-Felix reaction on testing sera from patients suffering from typhus in the totally different environment of Asia Minor. Moreover his work here made him think that the W-F reaction could be used not only for diagnosis but also for prognosis as he found that while cases of moderate severity gave a very high titre and an early response at about the 4th day, cases either of very severe or of very mild typhus showed on the contrary a much lower titre at a much later date, the reaction first becoming positive on the 7th day — (see Ztschr. J. Immunitatsf U Exper. Ther., 1917).

In January 1917 Felix rejoined once more his senior partner in Durazzo, where Weil was having a most unhappy time being made very miserable by his inability to sweat in that hot climate; on top of this disability, Weil, like every other person at his station, contracted malaria and was laid up for weeks. As no further progress was being accomplished on their beloved reaction, it seemed to them that any other place would serve their ambitions better than Durazzo, and succeeding in getting a transfer back to the Eastern Front, Weil and Felix moved with their laboratory eastwards following the German and Austrian Armies as these troops invaded the Ukraine, until by March 1918, they found themselves stationed in a town called Kheson, to the north of the Black Sea, near Odessa.

In another joint paper (and here I assume that Weil with his greater experience, superior knowledge in the field of bacteriology and more academic mind must have been the guiding light) these two workers added another and possibly greater scientific landmark, namely their observations on 'H' and 'O' agglutinogens which has proved so useful in classification of bacteria, diagnosis of diseases, and production of vaccines. Their work was published in the Wien. Klin. Woch. in 1917. And sometime later in the war, in yet another joint contribution, Weil and Felix extended their concept of 'H' and 'O' forms to bacilli of the typhoid-paratyphoid groups — (see Wien. Klin. Woch, 1918)

When the Armistice was signed in November 1918, Weil and Felix stayed close together, packed and loaded their laboratory equipment on cattle trucks and returned to Prague. Now this was no mean feat; it took them two very eventful months to get back to Prague, escaping death at the hands of the Bolsheviks on one hand, and also escaping from the wild bands of men in Hungary who were massacring the Jews. Both Weil and Felix were ill with enteric infection. Their luggage and baggage, suspected on occasions of containing firearms and ammunition, were nearly confiscated several times, and only escaped official destruction by vehement explanations that the cases contained highly infectious pathologic material from typhoid and typhus patients.

It is the irony of Fate to have to report, after all their labour and travail, the tragic loss of all their records, kept safe through so many near-disasters. A

completed manuscript giving details of all their work was prepared and left with Weil to be forwarded onwards to the publishers before their partnership was broken up after their war-time service. However it so happened that, after Weil's death which happened soon after, this important manuscript with all the relevant protocols for the years 1916-1920 disappeared completely and has never been recovered up to this day.

Weil's death occurred at the very early age of 43 — yet another investigator who died in harness in the course of research on typhus; Weil was an honest laboratory worker dedicated to his work. As his chief, Professor Bail, said of him 'he had no interests outside his work'. Born in Neustadte (Straz), Western Bohemia on the 17th April 1879, Weil had studied in Prague at the German Medical Faculty (there were two Medical Faculties in Prague at that time — German and Czech), where he graduated in 1903. Deciding to take up pathology as a career he worked for several months at the Pathology Institute of the Municipal Hospital in Berlin, and later returned home to work at the Institute of Pathology of the German Medical Faculty in Prague, which at that time was headed by Chiari. Soon however he must have been attracted to Bacteriology and Immunology for he succeeded in getting a transfer to the Institute of Hygiene. He was appointed Assistant Professor of Hygiene in May 1905, and ten years later was given the title of Extraordinary Professor of Hygiene, in May 1915; in 1920 he became Head of the Serology Department of the Institute, by which time he had published over 100 scientific contributions. Edmund Weil spent all his working life in his Institute, apart for him the fortunate interruption caused by the War Years 1914-1919, when he commanded an Epidemiological Laboratory of the Austrian Army Medical Services, and which in fact provided his imagination with ample scope and his fervent zeal for work with good opportunities for research; and indeed by the discovery of the Proteus X strains and subsequent happy application of the agglutination test to the diagnosis of rickettsial diseases Weil attained world fame.

It has been said that 'the Weil-Felix Reaction was one of the most outstanding medical achievements of World War I'; and it was indeed suggested that to honour Weil, the Proteus X strains should be called *"Proteus weilii"*.

Previous to his war work on typhus fever, Weil had done research work in association with his chief, colleague and friend Professor Bail; his main studies were on Bacterial Aggressins, on chicken-cholera, and on other problems mostly in the field of immunology. By 1911 he had already become well known for his work on the Wasserman Reaction as applied to the diagnosis of syphilis; in collaboration with a colleague, Viktor Kafka (a serologist and a psychiatrist), he proposed a diagnostic test for paralysis based on the increased permeability of the meninges and the disparity of the haemolysin titres between the cerebro-spinal fluid and the serum — this test was called the Weil-Kafka Reaction. When the Great War of 1914-1919 was over Weil devoted himself wholeheartedly to all aspects of typhus research, etiology, diagnosis, prophylaxis and so on; and it was while some rickettsial infected louse materal was being injected into laboratory animal by his fellow-worker Friedrick Brienl that a highly infectious aerosol was sprayed accidentally into Weil's eyes probably by a loose-fitting syringe needle. Weil rapidly developed a severe form of typhus and died in his own rooms at the Institute of Hygiene, on 15th June 1922.

Felix was indeed fortunate to have had such a tutor and collaborator. When their partnership began Felix knew very little bacteriology, by the time it ended he had acquired an international reputation (but let me add in haste a quotation from the great Pasteur 'chance favours the prepared mind'). In the 1920's, Felix who had been an ardent Zionist for many years emigrated to Palestine and worked first as a hospital clinical pathologist in Tel- Aviv, and later as chief bacteriologist to the Rotschild Hospital in Jerusalem. However the conditions for research in

Palestine were not really satisfactory and he came to England and joined the staff of the Lister Institute, where once more he made his mark in international bacteriology circles by adding another form to his previous two bacterial forms 'H' and 'O', namely the 'Vi' form associated with full virulence of those typhoid bacilli which possessed this surface antigen.

When Vi-phage typing was introduced in Canada by Craigie and Yen, Felix rapidly exploited this new technique, so much so that Felix's Laboratory was designated as the International Vi-phage typing centre. During my own Army Service he even managed to persuade the British Army Medical Service, to substitute his alcoholised Vi-antigen containing TAB Vaccine for the older heat-killed and phenol-preserved Leishman's TAB Vaccine. I remember in my early days in the Army that according to the last digit in our regimental number the odds received the phenolised vaccine, and the evens received the alcoholised vaccine while statisticians at their desks in Whitehall tried to find out which was really the better vaccine for the Armed Forces to adopt.

Felix was elected FRS in 1943, an honour which accorded him great satisfaction. He died suddenly and quite unexpectedly from an attack of coronary thrombosis on January 14th, 1956 while going to the Lister Institute for a reprint of a paper which he needed at that time.

Section 2. Stadium 1

Just as when motoring on a long summer vacation one gets the urge to interrupt the straight pre-arranged course of one's journey, and to turn down the narrow rolling reeling country lanes which appear invitingly and unannounced, with no mention at all on one's touring map (and rightly so, as some turn out to be culs-de-sac), in such a manner did I progress lazily along the main W-F Highway so to speak, with occasional side-way deviations which lead to such findings, hitherto completely unbeknown to me, as:—

(a) The XV stran — L. Selber (Z. Hyg. Infekrkr, p. 146) in 1927 showed that an ordinary *Proteus vulgaris* strain kept in a collodion sac in the abdominal cavity of a guinea-pig infected with typhus becomes agglutinable by typhus-fever serum. The strain that he employed was called XV and was found after the above treatment to remain agglutinable even after five years cultivaton. Cross absorption tests with typhus fever serum showed that agglutinins for XV were not absorbed by X19 and vice versa.

(b) The XL strain — As narrated by Felix (Trans. Roy. Soc. Trop. Med. Hyg., 1933) the story as I chanced upon it was as follows 'In February 1930, a strain of Proteus, apparently identical with X19, was isolated by Dr. Lima from the faeces of a child 4 years of age, living in the centre of the area where cases of Sao Paulo endemic typhus have continued to occur ever since the disease was first recognised in 1929. The child had had a history of some obscure illness shortly before the isolation of this Proteus culture, which had been diagnosed as a second attack of measles, since he had already passed through measles and scarlet fever. Dr. Lima himself consdered, 'the second attack of measles' to have been a mild attack of typhus and he therefore tested the child's serum against the known types of Proteus X strains; the serum gave a low but positive reaction against OX19, OXK, and the homologous Lima strain. The child's serum had no 'H' agglutinins for Proteus X strains, nor for the Lima strain, thus indicating the absence of any chronic infection with Proteus which causes the production of high titre 'H' agglutinins.

When this Proteus culture was received from Dr. Lima it was con-

firmed that the strain behave exactly like an X19 in biochemical characteristics. The two strains were also apparently identical by serological cross-agglutination with H and H + O antisera. A more detailed serological analysis, however, disclosed very distinct antigenic differences between the two strains. For convenience the Lima strain was designated Proteus XL and the symbols OXL and HXL are used for its O and HO variants respectively. The O antigen complex of strain XL differs from that of the genuine type X19 strains not only by its content of group antigen of OXK type of which the latter strains are devoid, but also by possessing the group antigen of OX2 type in considerably greater quantities than the Old World X19 strains.

(c) Also the Wassermann-Weil-Felix test — my own descriptive title to cover attempts by some of the early investigators to adapt the Wassermann Complement Fixation test to the serodiagnosis of Typhus, using the Weil-Felix Proteus X strains; thus Richard Wagner described how working with Proteus X19 as an antigen he obtained complement deviation with typhus serum. His results (Munch. med. Woch, 1917) were confirmed by the orthodox W-F reaction in all cases of typhus fever. The typhus sera gave a negative Wassermann reaction with the syphilitic antigen, while syphilitic and other sera used as controls gave uniformly negative results using X19 as antigen in the Wassermann reaction.

(d) In these golden days when immunology has become a major medical subject in its own right, with rapidly expanding knowledge of serum immunoglobulins (IgM; IgG) and locally produced immunoglobulins (IgA) such as those found in nasal secretion after influenza or copro-antibodies after cholera, I was amazed to discover in those early days for the W-F reaction, that the investigators experimenting with this reaction had already recorded positive W-F results with urine, and even with CSF of typhus patients (Ballner, F. and Finger, A. — Wien. Klin. Woch, 1917; Fuchs quoted by Weil and Felix — Deut. med. Woch, 1917). Thirty years later Fleck (1947) noted antigenic material common to proteus and rickettsial organisms in the urine of typhus patients which showed once again the affinity between these 2 entities. This substance may appear in the first days of the disease in an amount sufficient to give a precipitation reaction, and when injected into rabbits it gives rise to rickettsial and OX19 agglutinins (quoted in Manson's Tropical Disease Manual, 15th Edit., p. 225).

(e) And again to discover the use of blotting paper as a vehicle for sera used in diagnostic serology mentioned as far back as 1918! — when up to that particular day I had been obviously labouring under the misconception that the use of blotting paper was a very subtle logistic modern stratagem employed by present day epidemiologists to collect samples of sera from pygmies of the Congo Forest Belt or from the Indian tribes of the remotest villages along the Amazon basin — but see Diehl (med. Klinik, 1918). Is there truly nothing new under the sun?

(f) Typhus (Proteus X) Vaccine — somewhere along the trail I have a hazy recollection that someone somewhere proposed such a preparation. This procedure looks so obvious, especially to me who have worked in vaccine research and production, that I would have been really surprised and disappointed if it had been overlooked in those days when typhus was such a deadly destroyer of mankind especially at critical times when human populations were hit by famine, war, other pestilences and numerous such-like catastrophes.

Section 2. Stadium 2

It used to be said, and as far as I can tell it is still being said, that all paths lead to Rome and so it was that through one of these minor roads I found myself once more rejoining the W-F highway, only this time it became twice (well, one third more, to be exact) its former size. And this conjunction held some considerable fascination in its own right.

Since the original work was done by William Fletcher and J.E. Lesslar, my story will be excerpted with minor modifications from their historic papers (Bulletins from the Institute of Medical Research, Federated Malay States, No. 2 of 1925; No. 1 and No. 2 of 1926).

William Fletcher (1872-1938), Director of the Institute of Medical Research, Kuala Lumpur, worked with Stanton on melioidosis; other investigations of his included a study of the relation between rice and beri-beri; dysentery; enteric fevers. There was also Fletcher's Rabbit Serum Culture Medium for isolation of Leptospirae.

Also in the story was Sir John Megaw (1874-1958), who as a result of personal infection, took life-long interest in typhus. He classified the typhus fevers according to their vectors — louse, flea, tick, mite — preferring this classification to that by the W-F reaction, because, he stated, the W-F reaction did not distinguish between louse and flea-borne typhus, and in tick-borne typhus results were irregular. Professor of Pathology and Principal of Lucknow University, 1914; Director-General of the Indian Medical Service, 1930, he was co-author, with Sir Leonard Rogers, of the textbook on 'Tropical Medicine.

Wolbach wrote:

> "There is a sort of typhus in Malaya which differs essentially from the ordinary type by reason of its low infectivity, though its symptoms are identical with those of the classical form.
>
> We call this variant 'Tropical Typhus' because it appears to be more common in the tropics. Megaw drew attention to the occurrence in India of typhus-like fevers which are identical with or closely related to the spotted fever of the Rocky Mountains. This disease closely resembled the Tropical Typhus of Malaya with two notable exceptions; in the Indian disease a profuse rash was present, and the Weil-Felix reaction was negative, in the Malayan the rash is inconspicuous but the Weil-Felix is always positive.
>
> It by no means follows that this disease is not the same in the two countries. It is true the Weil-Felix was negative, but apparently the results were not checked by positive controls, and it is possible that the strain of *Bacillus proteus* which was employed had become inactive. Wolbach agrees that typhus fever, Rocky Mountain fever and tsutsugamushi should be classified as one group because they have clinical and pathological features in common; serologically however the blood in the last 2 diseases does not seem to give a positive Weil-Felix reaction. Between August 1924 and January 1925 in Kuala Lumpur, the capital of the Federated Malay States we were able to diagnose 18 cases of typhus, seven of which were in Europeans. As a retrospective diagnosis several puzzling cases which had been hospitalised in the preceding 2 months had also very probably suffered from the same disease — but the possibility did not occur to us at the time".

"Our diagnosis was made by employing for the Weil-Felix Reaction a culture of X19, which was given to us by Dr. Neave Kingsbury, a strain which had been supplied to the Bland-Sutton Institute of the Middlesex Hospital from the Lister Institute in 1921. The tests were made in Wright's (Sir Almroth Wright who had years before applied the agglutination test to the diagnosis of brucellosis) pipettes with emulsions of living cultures, and the results were read after 2 hours in the incubator at 37°C. According to most authors the reaction usually appears at the end of the first week but in our series of patients, using the Bland-Sutton strain of X19, it was obtainable at the end of the second week.

It is important that frequent control tests should be made with the strain of *B. proteus* which is being used; some are

more sensitive than others and subcultures of the same strains vary in agglutinability. The strain which we employed became more readily agglutinable after being subcultured almost daily for several months.

The Weil-Felix test has not been found to occur in any disease except typhus; however there is a flaw in the Weil-Felix test as it is carried out at present. The cultures used for the test in different laboratories appear to have been derived from different strains of *B. proteus*, or, if they were originally from the same stock, they have been greatly modified in their reactions and agglutinability by prolonged subcultivation. This diversity of the different strains, which pass under the name of *B. proteus X19*, makes it imperative to carry out careful control tests when working with an unknown strain. It is unjustifiable to conclude that a patient is not suffering from typhus because his blood fails to agglutinate a culture of which one has had no previous experience.

The strains of *Proteus Vulgaris* have been classified according to their power of producing indole. Van Loghem and van Loghem-Pouro have named the indole producing strains the 'Indolegenes' group, and those that do not produce indole they call the 'An-indolegenes' group; they further observe that these 2 groups can be separated by their serum reactions.

In order to elucidate the divergent results of the Weil-Felix reaction as applied on one hand to our cases in Malaya and on the other to those described by Megaw and other observers in India and elsewhere we investigated several strains obtained from various laboratories in different countries, that is to say,

(1) The first strain which first came into our possession (and which we used to diagnose our first cases in Malaya) was given to us by Dr. A.N. Kingsbury in 1924. He had brought it to the Malay States from the Bland-Sutton Institute in the previous year; and he informed us that it had been obtained from the National Collection of Type Cultures in 1921.

(2) Four strains supplied by Dr. R. St. John Brooks, the Curator of the National Collection of Type Cultures, that is to say strain: 67 and strain: 0 — both presented to the National Collection by Dr. J.A. Arkwright; the Warsaw strain — obtained for the National Collection by Dr. W.M. Scott of the Ministry of Health; and the Jerusalem strain — a strain left behind by the Germans on the Turkish evacuation of Jerusalem during the war.

(3) Strains: Sumatra — obtained from Dr. H. Verrost of Medan in Sumatra;
Acton — supplied by Lt. Col. W. Acton of the School of Tropical Medicine in Calcutta;
Parel — supplied by Lt. Col. F.M. Mackie, of Parel, Bombay; and
German — obtained from Lt. Col. W.F. Harvey, of the Central Research Institute of Kasauli.

With the sole exception of the first strain supplied by Dr. Kingsbury, all these strains ferment saccharose, and maltose, and produce indole in peptone water.

The difference between these 2 groups of strains becomes even more apparent by serological tests (although they do show some serological affinities to each other).

Hence we investigated still further the strain supplied by Dr. Kingsbury by testing it in parallel with other cultures belonging to the van Loghems'. An indolegenes group, namely No. 59 Elders and No. 60 Pneumo, and also with a dozen cultures isolated from the faeces of cholera patients — Dr. Kingsbury's strain was the only one to give a specific Weil-Felix reaction when tested against tropical typhus patients' sera.

Therefore from the beginning of July 1925 all specimens of blood sent to the laboratory for agglutination tests were examined with:

(a) an Indolegenes strain X19 obtained from the National Collection (either strain: Warsaw or strain 67),

(b) as well as with the specific An-indolegenes strain: Kingsbury.

Using this two-strains test it became clear that there are 2 groups of patients suffering from typhus fever:

(a) those in one group who gave a positive agglutination reaction with

strain: Kingsbury but not with the Indolegenes strains,

(b) the other group gives a positive agglutination test with the Indolegenes strains but not with the Anindolegenes strain: Kingsbury. For example:

Patient Mengah Singh No. 3

Day of Illness	10	16	20	36	46	62
Strain: Kingsbury	500	2000	4000	1000	100	30
Strain: Warsaw	0	0	0	0	0	0

Patient Chanam Singh No. 53

Day of Illness	19	26	37
Strain: Kingsbury	160	160	80
Strain: Warsaw	1500	600	300

Now as to the question Why? and When? did Fletcher and Lesslar get their fortunate inspiration to take a chance with the famous and vital Kingsbury variant of the Proteus X strains the following from Topley and Wilson's book (1936 p. 1458) in reference to their main theme detailed above may be of interest to the reader.

"Our brief survey may commence with the tropical typhus of Malaya which though chronologically not the first to be recognised, occupies a position of peculiar historical interest in the development of our knowledge of these diseases.

Fletcher and Lesslar in 1925-1926 studied the endemic typhus of the Federated Malay States and were puzzled to observe that the Weil-Felix reaction to the Proteus X19 was positive in some cases but not in others. Wondering whether their strain of Proteus X19 was losing its agglutinability, they decided to try a fresh strain that had been brought out by Dr. Kingsbury from the Middlesex Hospital. This strain it must be noted was reputed to be a strain of X 19 but undoubtedly it differed from this type antigenically — which was rather fortunate. (Yet one more instance I supposed of medical serendipity).

Further work by Fletcher in 1930-1932 showed that typhus patients whose serum agglutinated the old strain of OX 19 were to be found in urban areas, particularly in ships and storehouses, while those whose serum was agglutinated by the recently imported Kingsbury strain came from country districts especially where rank grass and scrub had grown up on land which had been cleared of jungle. Fletcher therefore referred to the first form of typhus as urban or Shop Typhus, and to the second type of typhus as rural or Scrub Typhus".

The Epilogue

ONE.

1920's "Though theoretically the demonstration of *R. prowazeki* in lice fed on typhus patients may be used for diagnosis of typhus, in practice it is far too lengthy a proceeding. The only diagnostic test of importance is the Weil-Felix reaction.

From Topley & Wilson's book 1929 edition, page 1209.

1930's. "The Weil-Felix reaction has now been employed on a large scale and has been found remarkably valuable. As with many other serum reactions used in diagnosis, experience of it has brought to light certain anomalies and limitations that were not at first realised. Many workers have doubted its specificity, and have brought forward evidence to show that the reaction may be positive during the course of other infections and even in healthy persons".

From Topley & Wilson's 2nd. Edit., 1936, p. 1450.

1940's. The three serological varieties of Proteus X, known as OX 19, OX 2, OX K are no longer the only reagents by means of which the serological diagnosis of the different varieties of typhus can be made. Suspensions of rickettsiae can now be obtained in relatively large quantities by growing these organisms in the yolk sac of the developing chick or in the lungs of infected rats and mice. Purified rickettsial suspensions are now being tried in agglutination and complement-fixation tests with typhus sera in various parts of the world and there can be little doubt that this work will lead to important advances in the serology of the typhus group. For the present, however, most pathologists must continue to rely on the agglutination test with Proteus OX antigens as the sole test available

for the routine diagnosis of cases of typhus fever."

From A. Felix, 1944. Report to the Medical Research Council.

1950's. From time to time Felix was very vexed by the unsatisfactory techniques used by many workers for the Weil-Felix test. The fault really lay with the writers of certain textbooks. After World War II, Felix stressed the necessity of standardising the W-F test and his recommendations were published in the Bulletin of the World Health Organisation, 1950, p. 637. His suggestion of international serum standards for OX 19, OX 2, OX K were accepted by the WHO Expert Committee on Biological Standardisation.

TWO

1960's. "The only serological test formerly available was the Weil-Felix reaction was by no means specific, for OX 2, OX 19 and OX K. The Weil-Felix reaction was by no means specific for cases of undulant fever, relapsing fever and rat-bite fever gave a positive agglutination reaction. Diagnosis is now made by agglutination of rickettsial suspension and by complement fixation; rickettsiae from the infected yolk sac of the developing chick embryo being used as an antigen. The indirect fluorescent antibody staining has been demonstrated by Goldwasser and Shepard."

From Manson's Tropical Diseases Manual, 16th Edit., 1966.

THREE

1970's "Serological investigations of rickettsial diseases. In this note on methods used in the serological investigation of rickettsioses, Capponi discusses the merits of complement fixation (CF), rickettsial micro-agglutination (MA) and indirect immunofluorescence (IMF). During the early stage of an acute infection serological responses demonstrable by the MA, IMF and CF methods are comparable, at a later stage and in chronic and inapparent infections the MA test alone may be positive. If only one method is to be employed, micro-agglutination is recommended".

From an abstract 1921/1970. Tropical Diseases Bulletin.

No originality, no rigid attention to sequence of events, no completeness, nor, dare I say it, absolute accuracy is claimed for this essay which has been culled from the fruit and labours of many workers in this fascinating field of truly international medical practice. It is enough that your curiosity has been aroused if you happen to be a younger man than I am or that your memories have been revived if you are one of the generation of medical men preceding my own, indeed some of the protagonists taking part in this Weil-Felix epic story may in fact have been well known to you.

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