In the Bacteriological Department of the Lister Institute some very interesting studies on virulence of *Bacillus typhosus* and resistance to "O" antibody have been carried out by Dr. A. Felix and Miss R. M. Pitt.

In previous work on the bacteriological action of serum Felix and Olitzki had shown that strains of *B. typhosus* and *B. paratyphosus A* which are highly sensitive to "O" agglutinins are also highly susceptible to the bactericidal action of serum. From this observation it seemed justifiable to assume that strains of *B. typhosus* of the sensitive type are less virulent than the non-sensitive or inagglutinable strains. To test this hypothesis Felix and Pitt selected the mouse as the experimental animal, as Grinnell, and Perry, Findlay and Bensted have shown that extremely regular results are obtained when mice are used for virulence tests with *B. typhosus*.

It is generally recognized that in primary cultures from typhoid excreta inagglutinable strains of *B. typhosus* may be met with that are "apparent" "O" variants. They are readily agglutinated by anti-"O" sera, and when sub-cultured on agar develop abundant flagella and are then agglutinated to full titre by anti-"H" sera. But as long ago as 1920, Weil and Felix described striking differences between different strains of *B. typhosus* as regards agglutinability by "O" agglutinins. Felix, in a subsequent paper, showed that between types of extreme sensitiveness and extreme non-sensitiveness the difference in agglutinability by "O" agglutinins may be as great as multiples of 10, 50, or even more; that this property is constant in individual strains; and that the well-known Rawlings strain belongs to the sensitive type.

In their experiments with mice Felix and Pitt employed only strains of two types: (1) Extremely sensitive, agglutinable; and (2) non-sensitive, inagglutinable. These were selected by preliminary agglutination tests. Types of intermediate agglutinability, which they state form the bulk of the strains of *B. typhosus*, were not used.

Felix and Pitt found as the result of this work that in the living state highly agglutinable strains of *B. typhosus* are of low virulence, while living inagglutinable strains are highly virulent. Both types of strain, when tested by all the known methods for detecting roughness, were found to be perfectly smooth. There was also no difference in their content of "H" antigen.

If the inagglutinable strains are killed by heat or by treatment with chemicals, the inagglutinability is lost and the suspensions are agglutinated.
by "O" serum to the titre limit. It makes no difference whether the "H" antigen is destroyed by the treatment for killing the organisms, or whether it is left intact. The resistance to "O" agglutinins is also suppressed by growing the cultures for twenty-four hours on agar containing phenol in a dilution of 1:900; and simultaneously the virulence of the cultures is reduced to that of the avirulent agglutinable strains.

The virulent strains have no capsule; it was thought that the inagglutinability might be due to some capsule or mucoid substance, but the presence of such a substance could not be demonstrated. The toxicity for mice of the killed virulent strains is not greater than that of the avirulent strains. Filtrates from broth cultures and extracts from agar cultures of the virulent strains do not increase the virulence of the avirulent strains nor do they produce any inhibitory effect on "O" agglutination. When suspensions of killed organisms are used for agglutination absorption tests, no antigenic difference is demonstrable between the two types.

Felix and Pitt consider that the results of their experiments clearly indicate that the mere presence of smooth "O" antigen does not completely define virulence. Some obscure property is required to render this antigen resistant to "O" antibody.

The unreliability of smooth characters of a growth of B. typhosus as an indication of virulence had been demonstrated by the work of Perry, Findlay and Bensted. They found that when smooth cultures of twelve recently isolated strains of B. typhosus were injected intraperitoneally into mice the minimum lethal dose varied from 50 to 200 million organisms. The strains of high virulence, viz., having a lethal dose of 50 millions, were not agglutinated in the living state by T.O. antiserum at 37° C., but when killed they were readily agglutinated by T.O. antiserum at 52° C. The strains with low virulence were agglutinated in the living state by T.O. antiserum at 37° C.; the lower the virulence the more marked was the agglutination of the living organisms. The killed organisms of low virulence were all agglutinated by T.O. serum at 52° C. to a titre similar to that of the organisms of high virulence.

They also described an intermediate strain (A) which had a smooth colonial appearance, was stable in saline and gave a negative reaction with Millon's test and the acriflavine test, and yet the living bacteria were agglutinated by T.O. antiserum at 37° C. to a high titre. The minimum lethal dose of this strain was 100 millions compared with 500 to 600 millions of the original rough Rawlings and 50 millions of the rejuvenated Rawlings.

From this point of view the history of the Rawling's strain, which was used for many years in the manufacture of anti-typhoid vaccine, is of particular interest. Perry and his collaborators found that the original Rawlings gave colonies with a granular surface and irregular outline; it was readily agglutinable in the living state by T.O. serum at 37° C., and the minimum lethal dose was from 500 to 600 million organisms. By certain methods of passage through mice the colonies changed and showed...
only a slightly granular surface, the living organism became inagglutinable by T.O. serum at 37° C., and the minimal lethal dose fell to less than 50 millions. When subsequently employed as a vaccine this passaged strain conferred approximately ten times the protection given by the original vaccine.

Perry and his collaborators considered that the greater antigenic value of rapidly passaged strains of the typhoid bacillus might be due to the presence of an antigen elaborated by the interaction of the organism and the body processes.

In further experiments with B. typhosus, reported in the July 28 number of the Lancet, Felix and Pitt found that the factor rendering smooth "O" antigen resistant to the action of the "O" antibody is itself an antigen separate and distinct from the long-established "O" and "H" antigens of B. typhosus. In their previous experiments they had shown that suspensions of inagglutinable or resistant strains when killed by heat or chemicals were agglutinated by "O" immune serum to titre limit and quantitative absorption tests with standardized suspensions showed that sensitive and non-sensitive strains did not differ in their content of the smooth "O" antigen. Furthermore, in rabbit immune sera prepared by inoculation of inagglutinable strains killed by heating at 60° C., no residual agglutinins for the homologous strain were demonstrable when the "O" agglutinin had been removed by absorption with an agglutinable strain. These results seemed to justify the statement previously made that the two types of strain did not differ in their antigenic composition.

In their latest work Felix and Pitt prepared vaccines of suspensions of twenty-four-hour agar cultures of inagglutinable smooth and agglutinable smooth strains, standardized by opacity and killed by heating for one and a half hours at 58° C., and injected them into mice. Twenty-four days later a test dose of 2 M.L.D. of their most virulent inagglutinable strain was inoculated intraperitoneally. Of 20 mice vaccinated with the inagglutinable strain 15 survived in one experiment and in another 17 out of 19 mice survived. But of 22 mice vaccinated with the agglutinable smooth strain only 1 survived. Control normal mice were all killed by the test dose. These results correspond to the findings of Perry and his collaborators with the original and the passaged Rawlings strain.

It was clear from these experiments that vaccines prepared from inagglutinable strains heated to 58° C. conferred a high degree of protection, whereas vaccines from agglutinable strains similarly heated were almost devoid of protective action. Felix and Pitt consider that their protection experiment indicates the presence in the inagglutinable strains of a separate antigen which withstands heating to 58° C. They demonstrated the presence of this antigen by agglutination experiments. Immunization of rabbits with living organisms of three inagglutinable strains and two intermediate strains, Watson and Mrs. S. (this strain was used by Ledingham and Arkwright in their experiments and is said to represent
the most common type of \textit{B. typhosus}), led to the production of an antibody which is absent from the sera of rabbits inoculated with living agglutinable organisms or inoculated with heated suspensions of virulent inagglutinable strains. Felix and Pitt propose to designate the new antigen as the "Virulence Antigen" and to refer to it as the Vi. antigen.

The Vi. antibody is capable of clumping the inagglutinable strains, whereas the "O" antibody is unable to produce this effect. The macroscopic appearance of the Vi. agglutination is very similar to that of "O" agglutination. Small granules of uniform size are formed which settle slowly and leave the supernatant fluid clear. The titre of the antibody is low; the highest Vi. titre observed was 1 : 400.

All the virulent strains reacted with all the sera containing the Vi. antibody, and the intensity of the reaction showed a definite relationship to the degree of virulence. Absorption tests showed that the living organisms of the highly agglutinable type of \textit{B. typhosus} were unable to reduce the Vi. titre. All the inagglutinable strains tested removed the Vi. antibody readily, irrespective of whether the "O" and "H" agglutinins had been simultaneously removed or reduced. Experiments on the neutralization of the so-called endotoxin of \textit{B. typhosus} indicated that "O" antibody has a definite neutralizing effect on the endotoxin, but Vi. antibody is incapable of this action.

The new antigen and its corresponding antibody would thus appear to be mainly concerned with the reactions that inhibit tissue invasion.

Felix and Pitt consider that in anti-typhoid inoculation the Vi. antigen must be taken into consideration and that to ensure the maximum efficacy of the vaccine the strain or strains selected must have the highest possible content of the Vi. antigen, in addition to the normal quota of "O" antigen. In this connection it is interesting to note that for some months the vaccine manufactured at the Royal Army Medical College has been prepared from strains of typhoid and paratyphoid organisms conforming to the types indicated.

Felix and Pitt also believe that serum containing both the Vi. antibody and the "O" antibody will prove to be of value in the treatment of typhoid patients.

Work on the cultivation of vaccinia virus on artificial media has been continued by Dr. Eagles. In cell-free fluid media he has completed a series of passages in which the washed elementary bodies obtained from deposits of Berkefeld V filtrates of dermal virus centrifugalized at high speed were used for the initial seeding; kidney extract, prepared by grinding, salting and high speed centrifugalization, constituted the culture medium. Seven generations of subcultures have been carried through without apparent loss of potency, the final subculture representing a multiplication of 20 times the original potency of the seeding as judged by animal titration.

Workers in other countries have not succeeded in securing evidence of growth of vaccine virus when cultivated in cell-free media, and Dr. Eagles
and Dr. Sabrin, U.S.A., are now working in conjunction in an effort to explain these conflicting results.

Dr. Eagles has inoculated washed elementary bodies from centrifugalized filtrates on a solid cell-free medium consisting either of chicken embryo or solid adult rabbit-kidney stiffened by the addition of hen plasma and a small amount of agar. No growth appeared on the surface of the inoculated medium though active virus could be obtained by surface washing up to seven days after inoculation.

Suspensions of elementary bodies, derived from filtrates of dermal virus by high-speed centrifugalization, are stated to possess high activity. As they represent the virus in the purest form and are sterile, and can be prepared with ease and economy, an investigation of their activity is being made under various conditions of storage. The survival periods of these suspensions in a medium of simple broth compare very favourably with those obtained with crude pulp in saline or glycerol saline.

Varicella and herpes zoster have for some time been considered to be related, and now it appears that Paschen has found elementary bodies similar in size and staining to those of vaccinia and varicella in the vesicle fluid of early skin lesions of zoster. Dr. Amies has obtained pure suspensions of the elementary bodies of herpes zoster by high-speed centrifugalization of the vesicle fluid. These suspensions are agglutinated by the serum of the convalescent zoster patient. This serum also agglutinates the elementary bodies of varicella to approximately the same titre. In a few cases varicella convalescent serum has also been found to agglutinate both varicella and zoster elementary bodies. The belief that the two viruses are closely related seems to stand now on a scientific basis.

A virus \((B.\ virus)\) has been recovered by Dr. Sabin from a fatal case of acute ascending myelitis in New York which is readily communicable to rabbits by intradermal injection, and produces in them an ascending paralysis accompanied by necrotic foci in certain viscera similar to those associated with highly virulent vaccinia and herpes infections. The virus in the rabbit brain and cord is very active, and a \(1:5,000\) dilution of a ten per cent highly centrifuged brain and cord emulsion produces paralysis and death after intradermal injection though the skin lesions may be minimal or almost imperceptible.

The \(B.\ virus\) is filtrable through Berkefeld V and N candles, and high-speed centrifugalization of the filtrate has yielded a highly potent sediment and a supernatant fluid entirely free of virus. It seems evident, therefore, that \(B.\ virus\) contains elementary bodies, and further investigation of its relation to vaccinia and the herpetic group of viruses is being actively pursued by Dr. Sabin at the Lister Institute. He has succeeded in infecting a rhesus monkey with rabbit virus by intracerebral and intraperitoneal injection, the animal showing marked paresia on the second day, followed
by death on the third day. The brain and cord from this monkey were
infective for rabbits, producing a large haemorrhagic skin lesion, ascending
paralysis and death.

In 1931 standards for vitamins were adopted, and for the past three
years workers at the Institute have investigated the following subjects:
vitamin A, pure crystalline carotene as a standard and the influence on
stability of the solvent used; vitamin B, the stability at different tem-
peratures of the standard absorption product on acid fullers' earth from
rice polishings; vitamin C, suitability of pure ascorbic acid as standard
in place of the fresh lemon juice previously recommended. A specimen of
coconut oil was found to be the most satisfactory solvent for use with
crystalline carotene. Recent researches have shown that there are several
isomeric forms of carotene. It has been found that pure β carotene dis-
solved in coconut oil has a deeper yellow colour and is biologically more
active in a somewhat smaller dose than the sample used as the Interna-
tional Standard.

Miss A. M. Copping has shown that copepod material, received from
the Marine Station at Plymouth, has a relatively high vitamin D content.
The dried material has been found to contain much more than 0·25 unit
vitamin D per gramme. This explains the rich deposit of vitamin D in the
liver of the codfish, which is stated to feed largely on copepods at certain
seasons of the year.

The examination of plant material for vitamin D has shown that the
vitamin which is abundantly formed when fresh green leaves are exposed
to powerful ultra-violet irradiation tends to disappear when the material is
kept, the rate of disappearance depending on the temperature. No evidence
was obtained of the presence of vitamin D in germinated wheat, contrary
to the statements of some workers that cereals develop vitamin D as well
as vitamin C in the process of germination.

At the request of the Accessory Food Factors (Vitamins) Committee,
the potency of ascorbic acid in relation to the vitamin C standard of lemon
juice has been evaluated. It has been found that the activity of 1 milli-
gramme of ascorbic acid is equivalent to 20 to 30 international units
(2 to 3 cubic centimetres of lemon juice).

Maize and wheat proteins in relation to the aetiology of pellagra have
been investigated by Mr. Hutchinson, working with Dr. Chick; he has
now completed his researches on the relative value of wheat and maize
proteins for supporting the growth of young rats. In the diets used, wheat
and maize proteins respectively formed the sole source of nitrogen, and,
when comparison was made only between experiments which correspond
exactly as regards the amount of protein and calorie intake, the value of
wheat protein was found to be only slightly superior to that of maize
protein. The difference was so small as to be of doubtful significance.
This work does not support the theory that endemic pellagra associated with the use of diets containing maize as the staple cereal can be explained by an amino-acid deficiency in such diets.

The association of pellagra with supposed toxic substances in maize has been investigated by Dr. Chick, Mr. Prunty and Miss Copping. They showed that rats and mice could be reared on diets in which ninety per cent of the calories was derived from maize products without the occurrence of any toxic symptoms, nor were any deleterious effects noticed when the animals consuming these diets were exposed to ultra-violet or visible light from artificial sources.

The toxic acid substance separated from maize by Stockman has been prepared from maize by Mr. Prunty and tested on animals. Only rarely were the paralytic symptoms described by Stockman observed, although large doses (0.5 to 2 milligrammes per gramme of body-weight) were given subcutaneously and intravenously to mice and frogs.
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