

STUDIES ON PLEUROPNEUMONIA-LIKE ORGANISMS:
THE L4 ORGANISM AS THE CAUSE OF WOGLOM'S
"PYOGENIC VIRUS"

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(With Plate II)

IN June 1938 the writer obtained three rats from Dr Gye and Dr Knox which had been previously infected with the "Pyogenic Virus" received by them from Dr Woglom (Woglom & Warren, 1938*a*). Pus from these rats was spread on plates of the writer's "special medium" for the isolation of pleuropneumonia-like organisms. No bacterial organism had been detected in the pus which, however, was full of granules and showed some filaments in the dark-ground preparation; after 6–10 days of incubation the plates were crowded with tiny colonies. They proved to be colonies of a pleuropneumonia-like organism, and the finding was recorded in an appendix to a recent paper by the writer (Klieneberger, 1938). At the edge of the growth from the seeded pus, where unhampered development was possible, the colonies showed a granular structure with a well-marked dense centre (see Fig. 1). No other organism was isolated from the lesions. More rats were now infected from the pus and all developed abscesses in the way described by Woglom & Warren (1938*a, b*). From these lesions the same culture was obtained (to be referred to as "Woglom strain I"). Since June 1938 up to date the pyogenic agent obtained from Dr Gye (to be referred to as "original agent") has been transferred from rat to rat by subcutaneous injection of the crude pus. So far, forty-five rats have been infected with this agent; they all developed abscesses, and the pleuropneumonia-like organism was invariably isolated from the pus. In thirteen of these rats spleen culture was attempted, and the pleuropneumonia-like organism was obtained from nine out of these thirteen spleens. If the spleen culture was positive, subcutaneous injection of a suspension of spleen produced an abscess, while a culturally negative spleen had no effect if injected.

INFECTION EXPERIMENTS WITH PURE CULTURES

The following were tested: (1) liquid culture of "Woglom strain I", (2) emulsified serum-agar growth of "Woglom strain I", (3) liquid culture of "Woglom strain I" plus sterile agar emulsion, (4) liquid culture of "Woglom strain I" plus sterile guinea-pig's brain emulsion, (5) serum-agar emulsion alone, (6) guinea-pig's brain emulsion alone.

If the liquid culture of "Woglom strain I" was injected subcutaneously a tiny node or abscess resulted; but if this primary node was ground up and suspended in broth or saline, and the suspension was injected into a second rat, an abscess of ordinary size developed; this could be passaged from rat to rat exactly as the "original agent". Subcutaneous injection of materials (2), (3), (4) reproduced at once the same effect as injection of the "original agent", while the control materials (5) and (6) produced no effect at all. The lesions produced by (1), (2), (3) and (4) were transferable from rat to rat exactly as the "original agent". From all these abscesses, produced in the first instance by subcutaneous injection of culture material, the same pleuropneumonia-like organism was recovered. There was only this difference, that the colonies from abscesses started with "Woglom strain I" grew up more quickly than those from the "original agent", because the organism had already been adapted to growth on artificial media. Even after a number of passages from rat to rat the organism did not lose this property. Though the amount injected subcutaneously into different rats varied sometimes within wide ranges the lesions developed usually in much the same way. Forty-four rats have been infected by subcutaneous injection of cultures or suspensions from abscesses started with cultures in the first instance. In ten of these rats the spleens were cultivated and eight of them yielded a growth of the pleuropneumonia-like organism.

If intravenously injected into young animals the liquid "Woglom strain I" culture had no effect at all; but if a pus suspension derived from an abscess produced by culture material was injected intravenously a very striking effect occurred. The rats became ill on the third or fourth day following the injection and developed oedematous swellings of their feet and other joints which impeded their movements badly. They did not gain in weight as compared with uninjected animals of the same litter. The following experiment with a litter of eight rats, 3 weeks old, all about 45 g., is described in some detail in order to demonstrate the effect of intravenous injection and its consequences.

After twenty-one passages of "Woglom strain I" on artificial media an abscess was produced subcutaneously by injecting an emulsion of a serum-agar culture into a rat. After two more rat passages of this agent a pus suspension was prepared from the last abscess. Six rats were given intravenous injections of this suspension, the remaining two rats being kept as controls. The six rats were taken ill on the third day after the injection; one of them recovered quickly, while the five others developed swellings of the two hind-feet; one of these five developed a third swelling in the shoulder joint, and a second animal developed an additional "lump" on the tail. Twelve days after the injection the weights of the two control rats were 85 and 88 g. respectively, while the injected rats weighed between 50 and 55 g. One of the infected rats was killed on the twelfth day after the injection, and the organism was recovered from all the different lesions. The remaining rats recovered during the next 10 days and the lesions cleared up gradually. 22 days after the first injection they were given a subcutaneous injection of the "original agent". While the control rats developed the usual abscesses the previously injected rats showed no reaction noticeable from the outside. When killed and dissected, small nodes were found at the site of the last subcutaneous injections; two of them proved to be only small thickenings, while three of them contained pus, the size of a pin's head. From this pus the pleuropneumonia-like organism was recovered.

A corresponding experiment in which—instead of the material derived from a culture—the “original agent” was used for intravenous injection, gave similar results. The oedematous swellings of joints which can be regularly produced by intravenous injections of pus suspensions (no matter if derived from a culture or from the “original agent”) do occasionally also occur in rats which have been inoculated subcutaneously. The liquid secreted into the joints may become transformed into pus.

The experiments described show that material derived from pure cultures is able to produce the same lesions as the “original agent” if subcutaneously or intravenously injected into laboratory rats. Following the injection a cross-immunity results which, however, is not complete. The experiments show further that the infectivity is greatly increased if a sterile agar emulsion or cells are injected with the culture. A small amount of the irritating agent (agar or cells) is sufficient to start the development of the lesions. The L 5 organism, the causative agent of “rolling disease” (Findlay *et al.* 1938), behaves in a similar manner in mice as the L 4 organism does in rats; the liquid culture is incapable of initiating symptoms when injected intracerebrally, but mixed with agar it produces the disease.

To make sure that the material received from Dr Gye and Dr Knox, and denoted “original agent”, had not changed its properties when transferred to English rats, the writer asked Dr Woglom for a sample of his “pyogenic virus”. He had so far not been able to obtain cultures from his material (Woglom & Warren, 1938*a, b*). In October 1938 the writer obtained, through Dr Woglom’s kindness, dried pus material which had previously been examined by him without yielding cultures. The material, labelled “41 A”, was ground up and emulsified in serum broth. One part of it was directly inoculated in liquid and on solid “special medium”; part was subcutaneously injected into rats. A number of characteristic pleuropneumonia-like colonies appeared on the solid medium after 10 days of incubation. The number was much smaller than if fresh material had been spread. From the liquid cultures plates were spread after a week of incubation; they yielded growth of characteristic colonies after about 6 days of incubation. The same result was obtained when the process was repeated with a remaining portion of the dried material that had been stored in the cold. The rats inoculated with “41 A” material developed abscesses in the same way as with the “original agent”. From the pus of these rats cultures were obtained not distinguishable from the “Woglom strain I” cultures or those obtained directly from the dried “41 A” material. It was easily proved that Dr Woglom’s “41 A” was in all respects identical with the “original agent” obtained in June 1938 from Dr Gye.

EXAMINATION OF STRAINS

During the course of this study, four strains of different origin were examined. The first culture, isolated in June 1938 from the “original agent”, was the “Woglom strain I”. It had undergone thirty passages up to January 1939.

The second strain, isolated from Dr Woglom's dried material "41 A", was called provisionally "Woglom strain II". It has undergone ten passages up to January 1939. Close observation of these two strains revealed a striking similarity with two other strains in the writer's collection of pleuropneumonia-like cultures, viz. the old strain "72 gland", described in 1938 as belonging to the L 4 species, and a strain, only recently isolated from a rat by the writer and called "rat 46". This latter strain was obtained from an "artificial abscess" produced by subcutaneous injection of an L 3 strain in agar emulsion. These mixtures produce pus in rats in the first instance, but cannot be transferred to new animals. This particular abscess however, proved, contrary to the rule, to be transferable. The discrepancy was cleared up when a culture was made from the pus, for it yielded a small number of L 3 colonies and a large number of L 4 colonies. As the L 4 had not been injected into this rat, a dormant infection must have been lighted up by the experimental injection. An L 4 colony was picked out and the new strain called "rat 46". This new L 4 strain "rat 46" and the old L 4 strain "72 gland" were used together with the two new strains "Woglom strain I" and "Woglom strain II" for a comparative examination.

Growth and morphology

The four strains are all of the same granular colony type. The centre of the colonies is dense and very well marked. In the peripheral zone large globules may sometimes be detected (see Fig. 2, which shows a well-developed 14-day-old colony of "Woglom strain II"). All four strains develop well in the liquid "special medium" in which they grow with slight uniform opalescence. The dark-ground picture reveals chiefly small elements and rarely clusters of globules. The strains proved to be filterable through Berkefeld V filters.

Serology

The four suspensions (1) "72 gland", (2) "46 rat", (3) "Woglom I" and (4) "Woglom II", were tested against the following nine sera prepared in the rabbit with cultures of the following: "72 gland", "46 rat", "Woglom II", organism of *pleuropneumonia*, organism of *agalactia*, L 1, L 3, *Asterococcus canis* I and *Asterococcus canis* II. The four suspensions under examination were agglutinated by the "72 gland", "46 rat", and "Woglom II" rabbit immune sera to a titre of 1:160 (see Klieneberger, 1938). The same four suspensions were negative with all the other sera of different species of the pleuropneumonia group. The agglutination tests show that the four strains under consideration are in all probability serologically identical and belong to the L 4 group. Absorption tests have not so far been performed.

Animal experiments

It has already been mentioned that the cultures "Woglom I" and "Woglom II" produced lesions identical with those caused by the "original agent"

and the dried material "41 A". "Woglom I" has been used for a large number of animal experiments to find out if it lost its virulence in passage. It was used three times to start abscesses in the way described, firstly after four passages, secondly after sixteen passages and thirdly after twenty-one passages on artificial media. Accordingly these three strains used to initiate lesions were called: "Woglom I 4", "Woglom I 16" and "Woglom I 21". The two strains Woglom I 16 and 21 showed a slight decrease in virulence if their effect in animals was compared with the effect of "Woglom I 4", but after a few animal passages the two former strains regained their original virulence and produced abscesses of the same size as those produced by the "original agent". "Woglom I 4" was passed four times from rat to rat, "Woglom I 16" was passed eight times in animals and "Woglom I 21" was passed ten times. In all forty-four rats have been infected with these materials originating from cultures. Animal experiments with the strain "rat 46" showed that similar lesions could be produced with this strain, and be transferred from rat to rat. Three passages have been carried out and six rats have been infected with this culture. The strain "72 gland" seemed at first to give doubtful results in animal experiments. When it was first isolated in October 1936 it was tested only by injection of liquid culture into rats, so that its pathogenicity for the laboratory rat escaped notice. It has since been re-examined and injected together with agar emulsions. Only very small abscesses were produced at first, and when the pus was reinjected into new rats it again gave rise to small abscesses, but as the rat passages were continued, the sizes of the abscesses increased gradually. Finally inflammation of the surrounding tissues also occurred. Now after twenty rat passages abscesses of medium size are produced, and if injected intravenously one out of four animals develops swellings of the joints. This shows that the old strain gradually increases in virulence by animal passage, though after the twenty transfers from rat to rat which have been performed up to date, it has not quite reached the virulence of the new strains "Woglom I" and "Woglom II".

DISCUSSION

The L 4 organism is the only species of the pleuropneumonia group that causes "pyogenic lesions" in rats. All the other species of the group have been tested by the writer during the course of this study by the "agar emulsion" method. If injected together with agar the pleuropneumonia-like strains from soil and water isolated by Laidlaw & Elford (1936) and Seiffert (1937) do not even produce any lesion in the first instance, and they cannot be recovered from the place of injection. The other species examined, viz. L 1, L 3, pleuropneumonia, agalactia, *Asterococcus canis* I and II, produce a more or less small amount of pus in the first rat, and they can mostly be recovered from these lesions after 4-7 days from the date of injection; later on they disappear. The lesions, even if the pus is transferred early, can never be passed on from rat to rat. By comparative study of the colony type, the growth in liquid media, the



Fig. 1. Photograph of the edge of a plate spread with pus from a "pyogenic virus" lesion after 10 days' incubation. The L 4 colonies have grown up between the pus cells. $\times 80$.

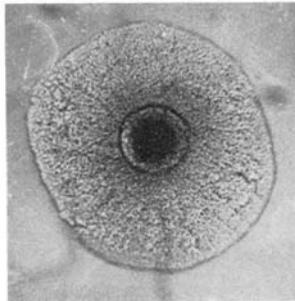


Fig. 2. Photograph of a well-isolated L 4 colony (strain "Woglom II") after a fortnight's incubation. $\times 80$.

serology and animal experiments, evidence has been brought forward that the four strains "Woglom I", "Woglom II", "rat 46" and "72 gland" are identical and representatives of the L 4 species. The L 4 occurs naturally in rats. It has been found twice by the writer in 1936 and 1938. Woglom, who was unable to isolate any organism from the purulent lesions, attributed the pathogenic effects observed to a virus agent designated by him "pyogenic virus". The "pyogenic virus" would now appear to be identical with the L 4 organism.

CONCLUSIONS

1. A pleuropneumonia-like organism has been isolated from all the lesions, produced by Woglom and Warren's "pyogenic virus" material.
2. The culture has also been obtained from spleens of infected rats and the disease was transferred only with positive, and not with negative spleens.
3. The characteristic condition of the rat described by Woglom and Warren has been repeatedly produced with different passages of strains isolated from the lesions.
4. Following infection with either "original agent" or culture material a cross immunity results.
5. The pleuropneumonia-like organism obtained from the "pyogenic virus" lesions has been identified with two other cultures previously isolated from rats by the writer. The three cultures belong to the L 4 group described by the writer in a previous paper.

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ADDENDUM

A private communication has been received by the author from Dr W. H. Woglom confirming the cultural and infection experiments here reported.

This has been received too late for inclusion in the discussion.

(*MS. received for publication* 8. II. 39.—Ed.)