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The Influence of Temperature on the Motility of *Pasteurella pseudotuberculosis*

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SUMMARY: Grown at 22° *Pasteurella pseudotuberculosis* was motile and had flagella and H antigen; grown at 37° it was non-motile and had neither flagella nor H antigen. The change occurred over a narrow range of temperature about 30°. Cells grown at 37° when transferred to 22° developed flagella without cell division; cells grown at 22° when transferred to 37° became non-motile, due to an unexplained adverse effect indicated by a high death-rate. It appeared that different metabolic processes were used at the two temperatures; it was not necessary to postulate the selection of a naturally occurring mutant and no evidence was obtained for the existence of an extracellular enzyme which would destroy flagella at 37°.

In addition to the 'short-term' effect there was a 'long-term' effect of temperature shown by a difference in degree of motility and rate of growth (at 22°) of cultures grown for several months at 22 or 37°. This may have been an extension of the 'short-term' effect, a period of training being required for the optimal functioning of the metabolic processes concerned. The long-term effect could be reversed by prolonged cultivation of the 22° substrain at 37° and vice versa.

Preliminary experiments indicated that virulence was affected by the temperature of cultivation.

A survey of the literature shows fairly general agreement that *Pasteurella pseudotuberculosis* is non-motile at 37° but motile at about 22° (Arkwright, 1927; Boquet, 1937; Bhatnagar, 1940). However, Boquet noted that two strains became permanently non-motile, even at 20°, after culture at 37° for several years; one of Arkwright's strains was slightly motile at 37°; Weitzenberg (1935) observed a few short granular flagella on organisms grown at 37°. Schütze (1932) showed that all four types of *Past. pseudotuberculosis*, based on somatic antigens, produced a common flagellar antigen at room temperature. Boquet claimed that a flagellar antigen was produced at 37°, but gave no convincing proof.

The object of the present work was to explain the mechanism by which the temperature of incubation affected the production of flagella.

MATERIALS AND METHODS

A single strain (134) of *Past. pseudotuberculosis* which had been isolated from a guinea-pig and dried was used for the main experimental work. By sub-culture twice a week for several months in Craigie tubes at 22° and on agar slopes at 37°, two new substrains were produced, referred to as 134/22 and 134/37. They were maintained similarly, the medium in each case being prepared from tryptic digest broth. The parent strain and the substrains were alike in not fermenting lactose or sucrose; they produced acid (without gas) from glucose and mannitol in 24 hr., and from salicin in 5-7 days. Indole was not produced.

The strain was pathogenic for mice and guinea-pigs by intraperitoneal injection of broth cultures but its virulence was low; subcutaneous injection produced a non-progressive local lesion which became sterile after a few weeks. It was not pathogenic for white rats.

Other strains were obtained from the National Collection of Type Cultures.

To detect motility broth cultures were examined in a 'hanging-drop'.

The *staining technique* for flagella was developed from a modification of Gray's method (Conn & Wolfe, 1938) and yielded clean preparations with deeply stained flagella:—

Stand new slides in concentrated nitric acid for at least 24 hr.; wash in distilled water; rinse in ethanol; pass them through the Bunsen flame until it turns slightly orange; cool gradually on an asbestos mat, to minimize breakage.

With a cool loop, transfer a little growth from an overnight agar slope culture to a few ml. of sterile distilled water; allow to stand for 5–10 min.; put a loopful on a slide, and spread with the edge of another slide; allow to dry without heating. By using the deposit from a centrifuged overnight broth culture (as suggested by Leifson, 1930), allowing this to stand in water for only 2–3 min. before smearing, a better result may be obtained. The agar slope, or broth, should be inoculated with motile organisms from a Craigie tube.

Apply cold mordant for 10–15 min.; wash well in running water; blot gently, and dry in the air without heating; apply cold stain for 7–10 min; wash, and blot dry.

Mordant. Mix 5 ml. saturated aqueous potassium alum, 2 ml. saturated aqueous mercuric chloride, 2 ml. 20 % (w/v) aqueous tannic acid, and 0.4 ml. saturated ethanolic basic fuchsin; allow to stand for 2 hr.; filter twice through Whatman No. 1 filter-paper and use immediately.

Stain. Heat 15 g. phenol with 3 g. basic fuchsin on a water-bath; when completely dissolved, add 30 ml. ethanol; mix thoroughly; add 170 ml. distilled water; leave for at least 24 hr.; filter 3 times through Whatman No. 1 filter-paper just before use.

RESULTS

Motility and flagellation. Broth cultures grown for 48 hr. at 22° showed a general turbidity and fine deposit, and contained short actively motile rods. Similar cultures grown at 37° had a granular deposit, a surface pellicle, and a few scattered floccules, but no general turbidity; they consisted of longer non-motile rods. Stained preparations of agar slope cultures grown at 22° revealed organisms with one to six peritrichate flagella, more commonly only one or two (Pl. 1, fig. 1). It was later observed that stained deposits of broth cultures showed more numerous flagella (Pl. 1, fig. 2) and this method alone was subsequently used for their demonstration. Organisms grown at 37°, however, showed no flagella (Pl. 1, fig. 3). There is apparently a gradual change (over a small range of temperature about 30°) from active motility to complete absence of motility (Table 1).

Both substrains 134/37 and 134/22 grew round Craigie tubes at 22° in 1 to 4 days, depending upon the consistency of the medium. At 37° the

Table 1. *Effect of temperature on motility, morphology and growth in broth*

Temperature	Appearance of culture	
	Macroscopic	Microscopic
25°	Turbid	80% actively motile: mostly short bacilli, single or in pairs
27°	Turbid	80 % actively motile: mostly short bacilli, single or in pairs
29°	Diffuse floccules	50 % slowly motile: many long bacilli and chains
31.5°	Deposit and few floccules	One motile cell seen (very sluggish) mostly long bacilli and long chains
34°	Deposit and few floccules	Non-motile: mostly long bacilli and long chains

growth of strain 134/37 was confined to the line of inoculation, whilst strain 134/22 produced a more diffuse growth in the upper part of the medium within the central glass tube, but never reached the surface outside. This inability to grow round a Craigie tube at 37°, even after 2 weeks' incubation in a tube which had been sealed to prevent evaporation, provides further evidence in favour of a complete absence of motility. The diffuse growth of strain 134/22 was obtained even when the Craigie tube was warmed to 37° before inoculation, the inference being that bacteria in the inoculum retained their motility for a time at 37° and spread some way into the medium where they grew. Such temporary maintenance of motility at 37° was again apparent in later experiments.

H antigen. Rabbits were immunized with flagellated and non-flagellated organisms grown at 22 and 37° respectively. The growth from 48 hr. cultures on blood agar plates was suspended in normal saline containing 0.1 % (v/v) formalin. After standing at 4° until sterile, the suspensions were diluted to about 5×10^8 organisms/ml., and stored at 4°. Each rabbit received 0.5 ml. suspension intravenously on three successive days in the first week, and three doses of 1.0 ml. in each of the second and third weeks. Samples of blood were taken at weekly intervals before, during, and after the course of injections, and the sera, preserved with 0.5 % (w/v) phenol, were stored at 4°.

Suspensions for agglutination were made from cultures grown at 22°. The 'H' suspensions were made by diluting twofold the one used for immunization. The 'O' suspensions were made by boiling saline suspensions for 2 hr. and diluting them to the same density. The instability of suspensions of *Past. pseudotuberculosis* from cultures grown at 37° was recorded by Arkwright (1927). We were able to produce a relatively stable suspension from such cultures by shaking before boiling, but the tendency to auto-agglutination was not completely removed and the agglutination end-points were consequently less distinct than with organisms grown at 22°. The titres of H and O agglutinins were obtained by using Dreyer tubes, held overnight at 56° in a water-bath. The results are shown in Table 2. Sera made against organisms grown at 22° contained both H and O agglutinins, whilst those against

organisms grown at 37° contained only O agglutinins. A similar absence of H agglutinin was found in the sera of rabbits immunized with a formalin killed broth culture grown at 37°.

Table 2. *Agglutination titres of rabbit sera against H and O antigens*

Serum:	...	...	A		B		C		D	
Antigen:	...	...	H	O	H	O	H	O	H	O
Immunized with organisms grown at										
Rabbit no.										
1	22°		0	0	5,000	500	10,000	2,500	20,000	5,000
2	22°		0	0	2,500	0	20,000	125	50,000	5,000
3	22°		0	0	125	25	2,500	1,000	10,000	2,500
4	37°		0	0	0	25	0	125	0	2,500
5	37°		0	0	0	0	0	250	0	1,000
6	37°		0	0	0	25	0	250	0	1,000

A, serum taken before immunization; B, serum taken after first series of three injections; C, serum taken after second series of three injections; D, serum taken after third series of three injections.

Although these results are highly suggestive of an absence of H antigen in organisms grown at 37°, confirmation was sought by the absorption of immune serum. Sera with high titres of both H and O agglutinins were pooled, diluted 1/5, mixed with equal volumes of very heavy suspensions of organisms grown for 3 days at 22 or 37°, and left in contact overnight at 22 and 37° respectively. The titres of these absorbed sera against H and O suspensions were as follows:

	H	O
Serum absorbed with living 37° suspension	10,000	< 25
Serum absorbed with formalized 37° suspension	10,000	1000
Control kept at 37°	10,000	2500
Serum absorbed with living 22° suspension	< 25	< 25
Serum absorbed with formalized 22° suspension	25 (tr.)	1000
Control kept at 22°	20,000	1000

Organisms grown at 22° absorbed both H and O agglutinins while those grown at 37° absorbed only O agglutinins. Treatment with formalin reduced the capacity of the organisms to absorb O agglutinin.

These experiments show that organisms grown at 22° were motile, possessed peritrichate flagella, gave rise to the production of H agglutinin in rabbits, and absorbed this antibody from immune serum. On the other hand, organisms grown at 37° were non-motile, possessed no flagella, did not cause the production of H agglutinin in rabbits, and were unable to absorb H agglutinin from immune serum. In each case there was complete correlation between the morphological and serological properties of this strain of *Past. pseudotuberculosis*.

There are several possible explanations for this difference associated with temperature:

(1) The establishment of different physico-chemical conditions in cultures at the two temperatures, e.g. different pH values.

(2) The production of an enzyme which at 37° would destroy the flagella or some substance essential for their synthesis (cf. streptococcal proteinase—Elliott, 1945).

(3) Mutation, and subsequent selection determined by the temperature of incubation.

(4) The use of different metabolic processes at the two temperatures. (Cf. amino-acid requirements of *Past. pestis*, Hills & Spurr, 1952.)

The influence of pH

Cultures in digest broth inoculated with motile organisms and incubated at 22 and at 37° were examined at intervals for motility, and the pH was estimated. The 22° cultures were actively motile; those grown at 37° showed only slight motility up to 24 hr. after which they were non-motile. At both temperatures, the pH showed an initial fall and subsequent rise (see Table 3). The changes in pH occurred faster at 37°, corresponding with more rapid growth, but otherwise they were similar at each temperature.

Table 3. *Changes in pH of cultures at 22° and at 37°*

Age of culture (hr.)	pH of culture grown at 22°	pH of culture grown at 37°
0	7.7	7.7
6	7.7	7.55
12	7.2	7.2
18	7.15	7.15
24	7.1	7.2
36	7.15	7.4
48	7.2	7.6
72	7.6	7.55

Each figure represents the average pH of two cultures.

In another experiment, digest broth was adjusted to pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5. Substrains 134/22 and 134/37 were each used to inoculate a series of tubes covering the entire range which were incubated at 22 and 37° respectively. Overnight cultures at 22° were all motile; those at 37° were all non-motile. These experiments leave little doubt that the differences between organisms grown at the two temperatures are not due to differences in pH.

Search for a destructive enzyme

If the failure to produce flagella at 37° were due to the action of a destructive enzyme, such an enzyme might possibly be demonstrated in filtrates of 37° cultures. Broth cultures of strain 134 grown for 18 hr. at 22 and 37° were Seitz-filtered. Samples of each filtrate were re-inoculated and incubated for 18 hr. at both 22 and 37°. Of these four cultures, the two which were grown at 22° (one in a 22° filtrate, the other in a 37° filtrate) were actively motile and showed no apparent difference in flagellation. Those grown at 37° were non-motile. This experiment did not eliminate the possibility of an enzyme which acted effectively only at 37° or of one which was adsorbed on to a Seitz filter.

Evidence against these possibilities was provided by observations on tubes of broth which were inoculated with motile cells from a Craigie tube culture of strain 134/22. After 1 day at 37°, these cultures contained a few motile cells and stained deposits showed occasional flagellated organisms some of which were faintly stained. After 2 days at 37°, the cultures were non-motile, but stained deposits still showed some flagellated cells, though these were all faintly stained and presumably dead. Considering the relatively rapid fall in viability of 37° cultures, revealed in a later experiment, it seems likely that these flagellated cells were part of the inoculum and that they died at 37° without destruction of their flagella. In view of the enormous preponderance of non-flagellated cells in these cultures the survival of a few flagella renders most unlikely the possibility that the non-flagellates produce an enzyme which can destroy flagella.

Although these experiments yield no evidence of an enzyme capable of destroying formed flagella, there remains the possibility of an enzyme which attacks at 37° some substance necessary for the formation of flagella. Such a mechanism would appear, however, to be closely related to the utilization of different synthetic pathways at the two temperatures, and this will be discussed later.

'Short-term' and 'long-term' influence of temperature

The problem was to determine whether a culture is motile at 22° as a result of the production of motile mutants, or whether a different metabolic process occurs at 22° which enables the bacteria to develop flagella. Some light was thrown on this problem by a study of the time-factor involved in the development of motility at 22° and the loss of motility at 37°.

Broth cultures of strains 134/22 and 134/37 were incubated at 22 and 37°. They were examined for motility after 1, 2 and 3 days, and on each occasion the culture grown at 37° was placed at 22° and re-examined 2 hr. later. The results are shown in Table 4. Strain 134/37 grown at 22° had a considerable

Table 4. *Motility of strain 134/22 and 134/37 at 22° and at 37°*

Strain	Temperature of			
	incubation	1 day	2 days	3 days
134/22	22°	90 % actively motile	50 % actively motile	50 % actively motile
134/22	37°	Occasional cell very sluggishly motile— rotating with little progress	Non-motile	Non-motile
	+ 2 hr. at 22°	More cells motile, and more active	Occasional cell rotating with little progress	Non-motile
134/37	22°	50 % actively motile (not as fast as strain 134/22)	50 % actively motile	50 % actively motile
134/37	37°	Non-motile	Non-motile	Non-motile
	+ 2 hr. at 22°	Non-motile	Non-motile	Non-motile

degree of motility after 24 hr.; strain 134/22 grown at 37° for 24 hr. had only a few sluggishly motile cells, probably from the inoculum.

In addition to this immediate or 'short-term' effect of temperature there was a cumulative or 'long-term' effect. Strain 134/22 grown at 37° developed some motility 2 hr. after transferring it to 22°, whereas strain 134/37 required longer. After 24 hr. at 22° strain 134/22 was more actively motile than strain 134/37.

The mechanism of the short-term effect

If the effect of temperature was to alter enzymic activity, it might be possible to demonstrate this in resting cells. Therefore broth cultures grown at either 37 or 22° until they reached the stationary phase were kept at the opposite temperature. Under these conditions changes in motility could be observed when there was little or no multiplication of the organisms. Viable organisms were estimated by counting colonies on blood agar by the method of Miles & Misra (1938); total counts were made in a Thoma blood-counting chamber. Microscopic clumps which formed in broth cultures at 22°, and to a greater extent at 37°, were broken up, without lowering their viability, by rapid shaking in a vibrator. Trials indicated that the error in the viable count was unlikely to exceed $\pm 15\%$, whilst that of the total count was probably less than $\pm 10\%$; these figures refer to the relative counts of different cultures, not to the absolute values of the total and viable counts.

Fig. 1. *a*, growth of *Past. pseudotuberculosis*, strain 134, in digest broth (total counts); *b*, growth of *Past. pseudotuberculosis*, strain 134, in digest broth (viable counts).

Digest broth cultures of strain 134 reached the stationary phase at 37° after 3–4 days; at 22° multiplication was active up to about 5 days but later was much slower (Fig. 1). An experiment based on these findings was made to test the effect of change of temperature on cells which were not dividing. Tubes of digest broth were inoculated with a motile culture from a Craigie tube. After incubation at 37° for 4 days (A) some tubes were transferred to 22° for 1 day (B); a control was incubated at 37° for 5 days (C). Other tubes were incubated at 22° for 5 days (D) and some of them transferred to 37° for 2 days (E); a control was kept at 22° for 7 days (F). Motility, flagella, total and viable counts are shown in Table 5.

Table 5. *Effect of change of temperature on cultures which have reached the stationary phase*

Culture	Time and temperature of incubation	Total count (million/ml.)	Viable count (million/ml.)	Hanging drop	Stained deposit
A	4 days at 37°	840	300	Non-motile	None flagellate
B	A + 1 day at 22°	800	320	About 30–40 % motile	About 30 % flagellate (peritrichate)—all these deeply stained
C	A + 1 day at 37°	840	280	Non-motile	None flagellate
D	5 days at 22°	990	625	About 40–50 % motile	About 70 % flagellate (peritrichate)—about one third of these pale
E	D + 2 days at 37°	880	100	Less than 5 % motile (sluggish)	About 70 % flagellate—most of these pale (deeply stained cells had not more than three flagella; most had none or one)
F	D + 2 days at 22°	960	500	About 40–50 % motile	About 70 % flagellate (peritrichate)—about half of these pale

About one-third of the bacteria in B became motile during 24 hr. at 22° without a significant change in the total or viable count. That this was a result of temperature is shown by C; cells which were non-motile at 37° became motile at 22°, a finding that would explain the temperature effect without recourse to mutation. The only other possibility was that the motile cells were newly formed as replacements for lysed cells, but there was no evidence to support this view: examination of hanging drops and stained films did not show any abnormal cells indicative of autolytic processes, and, moreover, many of the motile cells were long rods, the form usually found in cultures grown at 37°.

Conversely, cells which were motile when grown at 22° (D) became non-motile after a further 2 days at 37° (E), a change that did not occur at 22° (F) and was therefore a temperature effect.

This loss of motility when flagellated cells grown at 22° were kept for 2 days at 37° was associated with fall in viable count, inability of large numbers of cells to stain with normal intensity (Table 5) and, in addition, the presence of large numbers of poorly refractile organisms, 'ghost-cells' seen in hanging-drop preparations, indications of an adverse effect of a temperature of 37° on the viability of the cell in the medium employed. Loss of motility was not due to loss of flagella but probably was a manifestation, perhaps an early one, of conditions found in digest broth at 37°, not operative at 22°, which were unfavourable to survival of the bacteria. The nature of these conditions and the mechanism of their effect are not known. It is probable, however, that the factors which suppress development of flagella at 37° also bring about the loss of motility when motile cells grown at 22° are

kept at 37° and account for the high death-rate of bacteria at 37°. The temperature effect which suppresses the production of flagella or abolishes motility in flagellated organisms is not due to mutation; a more likely explanation is that certain metabolic processes are conditioned by temperature. Thus the short-term effects of temperature, both 22 and 37°, have a similar explanation; the metabolic processes at these two temperatures appear to be different.

It may be noted that motility in culture E grown at 22° and kept at 37° (Table 5) was not completely abolished and that some of the cells were deeply stained and had flagella. These cells were probably some which had developed at 22°; there was no evidence that new cells were produced in this culture at 37°. However the possibility cannot be completely excluded that a few flagellated cells may have been formed at 37°. A motile strain inoculated into a Craigie tube and incubated at 37° grew through the medium for a time; perhaps some motile cells retained for a time some stored-up material which enabled them to use their flagella at 37° and even develop a few new flagella but that this soon came to an end. Two-day cultures in broth at 37° were non-motile and 48 hr. cultures on blood agar at 37° had no detectable H antigen.

The development of flagella at 22° without cell division was also observed in nutrient broth, measuring density with a nephelometer. Cultures were inoculated with motile organisms, incubated for 2 days at 37°, and overnight at 22°. In one case about 15 % of the cells had become motile without increase in opacity; in the other there was an increased opacity of about 4 % but about 25 % had become motile. Similar results were obtained with four other strains of *Past. pseudotuberculosis* belonging to different serological types. Three further strains required four passages through Craigie tubes before becoming actively motile at 22°, and then gave similar results.

The long-term effect

A difference in the properties of the two substrains, induced by repeated subculture at 22 and 37° was demonstrated by comparing the rates of progress of their growth in Craigie tubes at 22°. Overnight broth cultures of the two substrains 134/22 and 134/37 of approximately equal density, were used. Each was grown at 22 and 37° and inoculated with a straight wire into five Craigie tubes which were incubated at 22°. All these tubes were similar in size. The progress of the growth down the central tube and up the outer part of the medium was recorded daily, the measurements of the five tubes of each series being averaged, as shown in Table 6 and Fig. 2. It is clear that the two substrains, as a result of prolonged cultivation at different temperatures, differed in their ability to grow and produce motile organisms at 22° and that this difference depended more on properties acquired by long-term cultivation than the conditions of the culture immediately preceding their inoculation. Substrain 134/22, accustomed to grow at a temperature suitable for motility, made faster progress through a Craigie tube at 22° than substrain 134/37, accustomed to grow at a temperature which precluded the formation of

flagella. This was so whether the culture used as inoculum was motile or non-motile.

The long-term effect was also demonstrated by a difference in the rate of growth in digest broth, using a nephelometer. Substrains 134/22 and 134/37 were equal at 37°, but at 22° substrain 134/22 grew considerably faster (Fig. 3*a, b*).

Table 6. *Rate of progress in Craigie tubes at 22°*

Group	Tube	Distance in mm. from point of inoculation				
		1 day	2 days	3 days	4 days	5 days
I	1	13	37	72 +	—	—
	2	15	38	62	70 +	—
	3	10	28	Broken	—	—
	4	17	34	64 +	—	—
	5	13	33	58	61 +	—
	Av.	14	34	64 +	—	—
II	1	10	29	57	68 +	—
	2	12	34	55	64 +	—
	3	13	34	62	64 +	—
	4	12	35	60	66 +	—
	5	9	24	54	65 +	—
	Av.	11	31	58	—	—
III	1	9	21	37	54	69 +
	2	13	25	39	59	73 +
	3	11	22	36	66 +	—
	4	8	21	31	60 +	—
	5	8	21	34	65 +	—
	Av.	10	22	35	61 +	—
IV	1	5	16	36	51	68 +
	2	6	35	47	67 +	—
	3	7	14	34	45	64 +
	4	8	20	35	62 +	—
	5	6	20	33	62 +	—
	Av.	6	21	37	57 +	—

I, inoculated with strain 134/22 grown overnight at 22° (motile inoculum); II, inoculated with strain 134/22 grown overnight at 37° (inoculum almost non-motile); III, inoculated with strain 134/37 grown overnight at 22° (motile inoculum); IV, inoculated with strain 134/37 grown overnight at 37° (non-motile inoculum); + indicates that the surface of the outer part of the medium had been reached.

Preliminary experiments have shown that the long-term effect is reversible. If substrain 134/37 is grown continually at 22° it assumes the properties described above for substrain 134/22; similarly, substrain 134/22 may be made to acquire the properties of substrain 134/37 by cultivating it for a period at 37°.

The probable explanation of this long-term effect is that it is a continuation of the short-term effect of temperature, the organism being trained, or becoming adapted, to use different metabolic processes more efficiently after repeated subculture. As the short-term effect can be explained apart from the gradual selection of a naturally occurring mutant, it would seem unnecessary to introduce such a postulate to explain the long-term effect.

Fig. 2. Progress of *Past. pseudotuberculosis* in Craigie tubes at 22°. I, inoculated from overnight broth culture of strain 134/22 grown at 22° (motile inoculum); II, inoculated from overnight broth culture of strain 134/22 grown at 37° (inoculum almost non-motile); III, inoculated from overnight broth culture of strain 134/37 grown at 22° (motile inoculum); IV, inoculated from overnight broth culture of strain 134/37 grown at 37° (non-motile inoculum).

Fig. 3. *a*, growth of *Past. pseudotuberculosis* in digest broth at 37°; *b*, growth of *Past. pseudotuberculosis* in digest broth at 22°.

Virulence for mice

Preliminary experiments have indicated that organisms grown in digest broth at 22° were more virulent for mice than those grown at 37°. This confirms one of the findings of Boquet (1937). It would seem unlikely from analogy that this phenomenon is related to the presence or absence of flagella. Further investigations are in progress.

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EXPLANATION OF PLATE₁.

Photomicrographs of *Past. pseudotuberculosis* stained to show flagella.

- Fig. 1. Grown at 22° on nutrient agar. × 600.
Fig. 2. Grown at 22° in broth. × 700.
Fig. 3. Grown at 37° in broth. × 700.

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N. W. PRESTON & H. B. MATLAND - MOTILITY OF *PASTEURILLA PSEUDOTUBERCULOSIS*. PLATE 1