Lethality for Guinea Pigs of Ultrasonic Extracts of *Pasteurella pestis*: its Relationship to Death of Guinea Pigs from Plague

A puzzling feature of studies on the basis of pathogenicity of *Pasteurella pestis* is the relationship of the behaviour of the so-called toxin[1–4] in guinea pigs to infection in the same host. This toxin is lethal for mice but not for guinea pigs[4,5], although the two hosts are equally susceptible to plague[1,6]. This anomaly led us to consider whether, in plague, guinea pigs are killed by a toxin different from that killing mice, or whether the 'toxin' as previously isolated is a degraded form of a native toxin that kills both species. The results described below support the latter view and give a reasonable explanation for the death of guinea pigs from plague.

Our work had two main innovations: (1) *P. pestis* and its extracellular products were obtained from infected guinea pigs and examined for any lethal component possibly absent from artificial cultures as was the anthrax toxin[7]. (2) The extraction of the organisms was milder and more complete than the saline extraction of acetone-dried organisms[3,8] or the autolysis of cells by prolonged incubation[1,2,4,5] hitherto employed.

Organisms were separated from the body fluids of guinea pigs which died from infection with *P. pestis* (strain *L*.37) by the methods described[9] for the collection of *Bacillus anthracis* grown *in vivo*.

The sterile filtered plasma from dying guinea pigs which originally contained $1–3 \times 10^9$ organisms per ml. had a small but variable toxicity for mice (*LD*50 > 1 ml.; 0·1 ml. given intravenously) and no detectable toxicity for guinea pigs (10 ml. given intravenously). For the following reasons, it was concluded that no purely extracellular toxin was produced *in vivo* by *P. pestis*, and that the slight toxicity of the plasma resulted from the liberation of intracellular material. First, the toxicity was absent or minimal if the plasma collected from a dying animal was rapidly cooled and filtered. Secondly, there was an increase in toxicity if the unfiltered plasma was stored at 0°.

Attention was turned to the organism as a source of toxin. Treatment with ultra-sonic waves proved the most satisfactory method of breakdown. Organisms grown *in vivo* were suspended in water (10^{10} per ml.) and subjected to ultra-sonic waves (Mullard Ultrasonic Generator, 2 megacycles per sec., 500 watts) at + 3° for 1 hr.; the organisms were mainly disintegrated and after centrifugation the extract was filtered through 'Millipore'.

Several batches of the filtrate (1 ml. = 1×10^{10} organisms) had a mouse (20 gm.) *LD*50 of 1/800 ml. (given intravenously) and when 2,000, 4,000 and 8,000 mouse *LD*50 were injected intravenously into guinea pigs (250 gm.) death-rates of 3/11, 8/10 and 5/5 respectively were obtained. Hence, the *LD*50 for a guinea pig is about 3,000 times larger than that for the mouse. On a body-weight basis, the resistance of a guinea pig to this toxic extract is about 250 times that of a mouse. This ratio is far smaller than the 5,000 indicated from the figures quoted by Schar and Meyer[4] for their plague 'toxin' and that indicated by the statement of Schar and Thal[5] that even in amounts of 300,000 mouse *LD*50 the plague 'toxin' never exerts a lethal effect on guinea pigs.

It seems, therefore, that the same toxin can be responsible for the death of guinea pigs and mice. The species difference in susceptibility of 250-fold is of the same order as that obtained with some other toxins[10]. In both mice and guinea pigs the toxin acted quickly; deaths occurred mainly within 12 hr. with clinical signs of shock and the presence of exudate in the body cavities. Furthermore, the *LD*50 of our toxic extract for guinea pigs was obtained from approximately 4×10^{10} organisms, which number is less than the total *P. pestis* organisms (at least $5–16 \times 10^{10}$) present in a guinea pig (250 gm.) killed by plague.

The properties of the toxic extract described above raise doubts as to whether the toxin as previously isolated is the true toxin of *P. pestis*. This is underlined by the finding that similar extracts have now been obtained from organisms grown *in vitro*. Thus, ultra-sonic extracts of organisms obtained from shaken cultures (28°) of *P. pestis* (strain *L*.37) in a medium similar to that of Higuchi and Carlin[11] (30 hr.), and another medium used by Davies[12] (40 hr.) killed, respectively, 2/4, 3/3, 1/1 and 2/8, 3/12, 7/8 guinea pigs (250 gm.) when 2,000, 4,000 and 8,000 mouse *LD*50 were injected intravenously. It appears, therefore, that *P. pestis* contains a toxic complex which hitherto has only been isolated in part due to deficiencies in previous methods of extraction.

The continued use of ultrasonic waves to form more complete extracts of *P. pestis* than hitherto examined may well shed light on other chemical aspects of the pathogenicity and immunity to plague. Already the technique has been the means of separating *P. pestis* grown *in vivo* into two constituents, namely, the highly toxic soluble material which appears to contain very little protective antigen and a residue which readily immunizes guinea pigs.

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Enhancement of Mouse-virulence of Group *A* Streptococci

The virulence of organisms can be enhanced by a variety of substances, mainly of carbohydrate nature (see review by Olitzki[1]). Current interest centres on mucin, which was found by Smith and his co-workers[2–4] to owe its activity to the synergic action of three factors—viscosity, a particulate residue and a heparin. The present communication shows that bacteriological peptone and some of its hydrolysis products also have considerable virulence-enhancing activity.

It was first observed that the minimal lethal dose (*LD*100) of group *A* streptococci injected intra-