avec le phage ϕD est toujours accompagnée par la répression de l'hémolysine β (conversion).

Les phages ϕRE et ϕD ont été isolés de la souche BSON de *S. aureus* qui est coagulase-positive et inductible par la mitomycine ou par les rayons ultraviolets[6]. Ces phages donnent deux types différents de plages sur la couche continue de la culture de la souche BSS, ensemencée directement sur la gélose BHI (Brain Heart Infusion, Difco). Le phage ϕD donne une plage de $^1/_2$ mm de diamètre, ronde, avec une culture secondaire au centre macroscopiquement visible. L'autre type de plage, approximativement deux fois moins grande, ronde, plus claire et dépourvue d'un centre opaque, correspond au phage ϕRE.

Les phages en clone pur ont été isolés par la méthode recommandée par NICOLLE[7], légèrement modifiée. Pour être certains d'avoir entre les mains le phage de lignée pure, nous avons opéré trois prélèvements successifs à partir d'un même type de plage bien séparée. Par la même occasion, nous avons comparé le nombre total de PFU[8] d'une plage à centre lysogène avec celui d'une plage claire. Une plage du phage ϕRE donne 10^8 PFU, approximativement deux fois plus qu'une plage à centre opaque.

Le phage de lignée pure a été régénéré sur les BSS. La propagation sur milieu solide donne un lysat phagique d'un titre plus élevé (ϕRE $> 10^{11}$ PFU/ml, ϕD $> 10^{10}$ PFU/ml) que celui obtenu lors de la propagation en milieu liquide. L'étude en microscopie électronique de ces préparations phagiques purifiées par centrifugation différentielle[9] a montré que le virion le plus grand, à tête ovale, queue rigide, plaque terminale massive de lysat phagique ϕRD (réf. [6], Figure 2) correspond à la plage claire dépourvue d'un centre opaque, et que le phage le moins grand possèdant une tête polyédrique et une queue délicate et flexible correspond à la plage avec centre lysogène.

Il est possible de lysogéniser la souche BSS avec chacun de ces phages à part ou avec tous les deux simultanément ou successivement. La lysogénisation du BSS par le ϕD est toujours accompagnée par la répression de l'hémolysine β et d'une augmentation de la production de la staphylokinase. Puisque également toutes les colonies β^- de la culture secondaire du BSS dans l'expérience de lysogénisation avec le ϕD se sont avérées lysogènes pour le ϕD, il s'agit d'une conversion. La synthèse de l'hémolysine δ n'est pas affectée par le ϕD. Par contre, la lysogénisation du BSS par le phage ϕRE peut être accompagnée à basse fréquence ($< 1/500$) de la perte de l'hémolysine δ. Le phage ϕRE à l'état de prophage dans la souche BSS n'influence pas la synthèse de l'hémolysine β. L'ordre de lysogénisation avec les ϕRE et ϕD lors de la lysogénisation double du BSS ne joue pas, semble-t-il, un rôle important.

Summary. The effects of double, simultaneous, or successive lysogenisation of BSS strain of *S. aureus* by two staphylococcal phages, both isolated from the same strain, have been studied. These phages (ϕRE and ϕD) are easily distinguished electronmicroscopically by the morphology of the virions as well as by the aspect of the plaques on the BSS. Lysogenization of BSS by ϕD is always accompanied by repression of β-haemolysin (conversion), but lysogenisation by ϕRE is only rarely accompanied by the loss of δ-haemolysin (the frequency of the phenomenon suggests a transduction). In double lysogenization of BSS strain by ϕRE and ϕD, the sequence of lysogenisation does not, apparently, play an important role.

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A Comparison of the Development of *Nippostrongylus brasiliensis* Larvae of Various Ages Introduced Orally and Subcutaneously into Laboratory Rats

In recent years some attention has been focused upon postembryonic development and behaviour of *Nippostrongylus brasiliensis* in laboratory rat. Host age, sex and immunity are a few experimental approaches employed to understand fully the mechanisms involved in the development of the parasite and its effect on the host (CHANDLER[1,2]; TWOHY[3,4] and HALEY and CLIFFORD[5]). SIMAREN[6] and SIMAREN and FABIANEK[7] using a single strain age reported that migration and development of *Nippostrongylus* larvae into adult worms was higher in the s.c. infected rats, and progressively grew smaller in the i.v., i.p. and orally infected rats. So far, no published data on the comparison of growth of the different stages of *Nippostrongylus* larvae introduced orally and s.c. into a single strain of rats. This report emphasizes the differences that occured between the various larval stages used, their efficiency and the effect of quantified infectivity of those stages in rats.

Materials and methods. The strain of *N. brasiliensis* used has been maintained for 2 years through a bi-weekly transfer to albino rats in the Parasitology laboratory at the University of Ife (Ile-Ife). The techniques used to culture the larvae, maintain stock of infections, inoculated experimental animals and recover adult worms were modified after that of YOKOGAWA[8] and HALEY[9]. The cultures were kept in a fly-proof cabinet in an air-conditioned room (temperature 23–37 °C). Larvae from 6-, 9-, and 12-day-old cultures were isolated, concentrated by low speed centrifugation and washed 4–5 times with 0.85% NaCl. 6–7-week-old laboratory bred albino rats completely free of parasites were infected, each receiving 800 larvae quantitatively determined with a disecting binocular microscope. All inoculated rats were kept in individual cages to avoid contamination.

Three experiments of s.c. infection were performed by injecting rats near the posterior neck region beneath the

skin. The first 6 rats injected 800 6-day-old larvae serves as the standard control; a second set of 6 rats with 800 9-day-old larvae and a third with 800 12-day-old larvae. For oral infection 3 similar experiments were carried out by introducing the larvae into the stomach of each rat directly from a small syringe without the need. This was followed by giving the rats a few drops of water to ascertain that all larvae given were swallowed. The number of eggs passed daily for 15 days from every 24 h fecal collection was determined by a modification of Stoll's[10] dilution egg-count method. Some rats from each experiment were starved overnight with the exception of water prior to necropsy to remove extraneous materials, permit cleaner and easier examination, collection and counting of adult worms in the intestine. The heart, trachea, lungs, and abdominal cavities were checked for worms. The length of male and female worms recovered from the experiments were measured and compared.

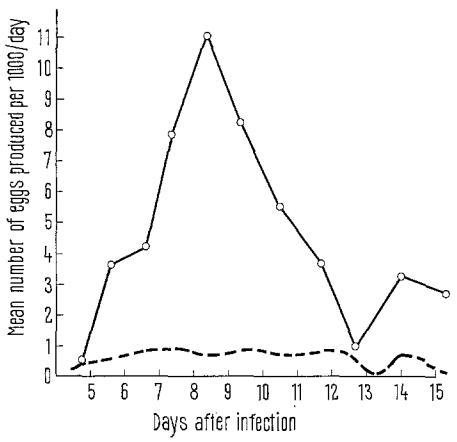

Fig. 1. Comparative egg count curves on rats infected s.c. and orally with 6-day-old larvae of 800 *N. brasiliensis*. ○—○, s.c. route; ---, oral route.

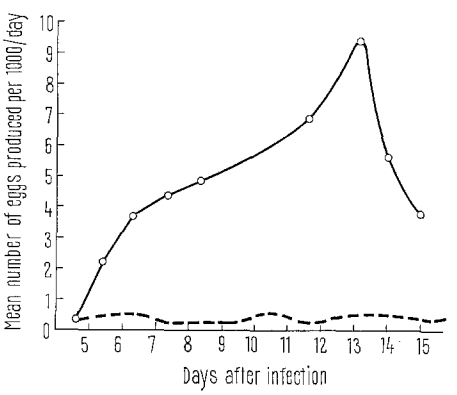

Fig. 2. Comparative egg count curves on rats infected s.c. and orally with 9-day-old larvae of 800 *N. brasiliensis*. ○—○, s.c. route; ---, oral route.

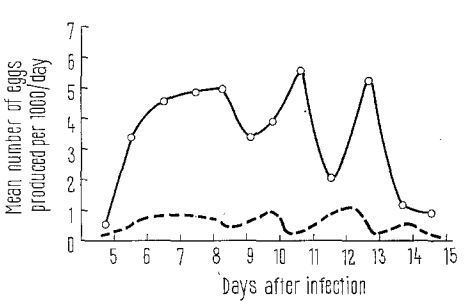

Fig. 3. Comparative egg count curves on rats infected s.c. and orally with 12-day-old larvae of 800 *N. brasiliensis*. ○—○, s.c. route; ---, oral route.

Results and discussion. The course of infection was followed by daily egg count, patent and prepatent period of the worm development, worm count, size and range of recovered worms, and percentage of larvae reaching adulthood. In the s.c. and oral experiments, all rats infected showed similar prepatent period of 6 days regardless of the varied age. The s.c. infected rats indicated a patent period of the 6th to 15th day while those orally inoculated extended to the 12th day. Rats infected s.c. and orally with 6-day-old larvae revealed their egg production peak on the 9th day post infection. Delayed peak in other groups infected with 9- and 12-day-old larvae appeared on the 11th to 13th day. The total number of eggs passed throughout the patent period were 54,922; 47,522, and 28,634 for the 6-, 9- and 12-day-old larvae in the s.c. infection. For oral infection, 1529; 1417 and 3189 eggs were produced for the 6-, 9- and 12-day-old larvae.

More female than male worms were recovered from s.c. infections (260, 186, and 129 worms for 6-, 9- and 12-day-old larvae) compared with 3, 2 and 2 worms in oral infection for similar larval age. Measurements of recovered adult worms from the s.c. and oral experiments were 2.49–4.06 mm (male length) and 4.06–5.2 mm (female length). Significant differences occurring in egg production and the number of matured parasites recovered from the intestine correlate with the increase of larval age used in the investigation. The reduced total egg output and worm counts strikingly indicate a decrease in infectivity with a corresponding increase in larval age. This confirms a parallel report for *Necator americanus* in man (Paye[11]), *Haemonchus contortus* (Roger[12]) and *Ascaridia galli* (Elliot[13]) and suggests that the defficiency in development might be due to a slow built up of an acquired immunity by the host to this parasite. The comparative egg count curves (Figures 1, 2 and 3) similarly support the differences in the efficiency of the infective larvae. However, these results provide new and useful information on the necessity of a specific route of migration, age of larvae and development.

Résumé. Chaque rat infecté reçut 800 larves et la totalité des œufs produits chaque jour dans les rats infectés par voie sous-cutanée fut plus grande que lors d'une infection orale. Dans toutes les infections orales et sous-cutanées, la production des œufs et le nombre des vers alla en diminuant, mais l'âge des larves augmenta. Ni les voies d'infection, ni l'âge des larves n'affectèrent la longueur des vers adultes qu'on récupéra.

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