bei 37 °C inkubiert, filtriert, und die gefärbten Zellkerne werden in der Bürker-Türkschen Kammer gezählt[4].

Differentialzählung der Exsudatzellen. Material von dem der Epidermis zugewandten Teil des Sephadexherdes wurde ausgestrichen, mit Hämatoxylin-Eosin gefärbt und der prozentuelle Anteil der Granulozyten bestimmt.

Ödemmessung. Das Pfotenödem der Ratte wurde nach subplantarer Injektion von 0,2 ml Sephadex bzw. $CaCl_2$-Sephadex volumetrisch gemessen.

Ergebnisse. Nach 6 Stunden unterscheidet sich, gleichgültig welche $CaCl_2$-Konzentration verwendet wird, der histologische Befund nicht von der Kontrolle. Nur bei hoher Ca^{2+}-Konzentration (30 mmol) sind hier um den Entzündungsherd auch Nekroseerscheinungen sichtbar. Zahl und Anteil der intaktgebliebenen Mastzellen ist bei hoher Ca^{2+}-Konzentration reduziert, da Ca^{2+} unter anderem auch Mastzellen zerstören können. Die Gesamtzahl der Exsudatzellen und deren prozentueller Gehalt an Granulozyten unterscheiden sich ebenfalls nicht von der Kontrolle (Tabelle). $CaCl_2$-Sephadex verursacht mit 30 mmol Ca^{2+} eine gleiche, mit 4,5 und 7,5 mmol Ca^{2+} eine stärkere Volumenzunahme der Rattenpfote als NaCl-Sephadex.

Auch nach 24 Stunden unterscheidet sich das histologische Bild, gekennzeichnet durch ein beträchtliches Restödem und reichlich Zellen um und im Sephadexherd, nicht von der Kontrolle, abgesehen von deutlicher werdenden Nekrosen. Die Gesamtzahl und der Anteil intakter Mastzellen ist vermindert. Das Verhältnis Granulozyten zu mononukleären Zellen im Exsudat hat sich, im Gegensatz zu dem nach 6 Stunden erhobenen Befund, verschoben: Während bei der Sephadexentzündung 86% der Zellen Granulozyten sind, wurden nach Injektion von $CaCl_2$-Sephadex (4,5 mmol Ca^{2+}) nur 60% Granulozyten gezählt. Dieser Prozentsatz nimmt mit steigender Ca^{2+}-Konzentration wieder zu. Die Zahl aller Exsudatzellen ist geringfügig niedriger als bei der Sephadexentzündung. Die Volumenzunahme der Rattenpfoten ist auch noch nach 24 Stunden beträchtlich: Während die Pfote nach Injektion von NaCl-Sephadex um rund die Hälfte ihres Ausgangsvolumens zugenommen hat, vergrössern sich die Pfoten durch $CaCl_2$-Sephadex um das Doppelte.

Diskussion. Aus den Versuchen geht hervor, dass eine erhöhte Ca^{2+}-Konzentration am Ort einer sich entwickelnden Entzündung keinen hemmenden Einfluss auf den Entzündungsprozess hat. Dies zeigen vor allem die nach 6 Stunden erhaltenen Ergebnisse. Eine «gefässabdichtende» Wirkung hätte sich gerade in diesem für die Entstehung und Ausbreitung des Ödems und für die Leukozytenemigration massgeblichen Zeitraum bemerkbar machen müssen.

Wenn Ca^{2+} überhaupt eine antiexsudative Wirkung haben, dann könnte diese durch Catecholamine zustande kommen, die durch intravenöse Injektion von Ca^{2+} freigesetzt werden[5].

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Comparison of the Ototoxic Effect of Kanamycin on Albino and Pigmented Rats, Studied Using an Operant Method[1]

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Summary. A study was made of the effect of daily administration of kanamycin (400 mg kg^{-1}) on the hearing of Wistar albino and Lister hooded (pigmented) rats, which had been conditioned to discriminate an acoustic signal. In all animals except one, the drug caused severe, permanent hearing impairment and there was no difference between albino and pigmented rats in onset or degree. Other work has suggested a mediatory role for melanin pigment in such drug ototoxicity but the significance of this must be questioned in view of the failure to find any differences in functional deficit.

Kanamycin is one of a number of antibiotics which exert a toxic effect on the ear and whose administration, in certain circumstances, may result in deafness. The elucidation of the manner in which such drugs cause deafness has attracted much interest and considerable effort, but the exact mechanisms remain unresolved.

Ototoxic drugs have been shown to accumulate in the inner ear and this has been attributed[3] to their affinity for melanin pigment which, in the cochlea, is found mainly in the stria vascularis. The implication that pigmented animals would accumulate ototoxic drugs in the inner ear to a greater extent than albino animals and would consequently be more likely to suffer hearing impairment has been investigated[4]. The hearing of albino and pigmented rats and guinea-pigs was assessed by measurement of acoustic startle reaction and a progressive diminution of startle reaction following ototoxic drug administration was taken to be indicative of the development of hearing impairment. Although preliminary results did suggest that drug-induced deafness occurred more readily in pigmented animals, subsequent studies were unable to reveal any significant differences between the ototoxic effects of kanamycin or neomycin on albino and pigmented rats and guinea-pigs. However, it was suggested, at the time, that the methods employed might not have been sufficiently sensitive to detect any subtle differences.

A further study has now been carried out on the effects of kanamycin on albino and pigmented rats, using an operant conditioning technique. It has been shown[5] that this is an appropriate technique for the study of the

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onset, development and relative extent of hearing impairment in rats, administered chronically with kanamycin.

Materials and methods. A detailed description of the apparatus and methods, including the training procedures, appears elsewhere[5].

Six male Wistar albino and 6 male Lister hooded (pigmented) rats were conditioned to discriminate an 8 kHz tone (56.5 ± 0.5 dB, *re* 0.0002 dyn cm^{-2}). The animals, 4 months old at the beginning of the study, were reduced by means of food deprivation to 85% of their free-feeding weights and were maintained at this level by provision of a daily weighed food ration.

Two operant test chambers, in sound-attenuating boxes, were used and discrimination of the tone was indicated by a differential rate of responding (lever pressing) between alternating tone and no-tone periods. The reinforcement schedule used to promote a higher rate of responding during tone periods is shown in Figure 1. The animals were trained in trials lasting 45 min until their discrimination performances (DP's) stabilized. When baseline DP's were established, daily (except Sat and Sun) s.c. injections of kanamycin (400 mg kg^{-1}) were given

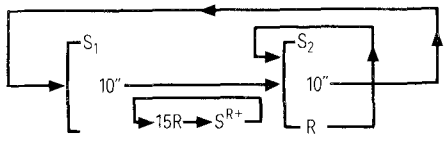

Fig. 1. Reinforcement schedule used to promote a differential between rates of responding in the tone and no-tone periods. S_1-tone, S_2-no-tone, R-response, S^{R+}-reinforcement. S_1 is a fixed interval of 10 sec. In the presence of S_1 every 15th response is reinforced with a food pellet. S_2 is also a fixed interval of 10 sec in the absence of any response. S_2 is replaced by S_1 at the end of this 10 sec, although each response during S_2 delays the onset of tone (S_1) by a further 10 sec.

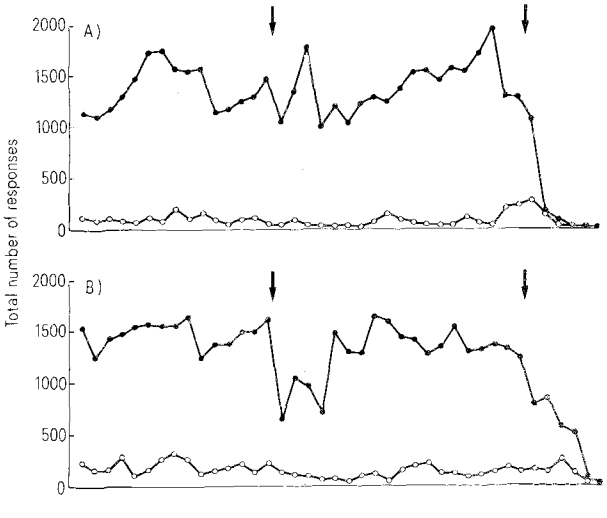

Fig. 2. Tone discrimination performances of an albino rat (A) and a pigmented rat (B) showing responses during tone (●—●) and no-tone (○—○). Each point is the mean of 2 consecutive trials. This simplified the presentation of results and was considered to be justified in that evanescent fluctuations in discrimination performances were not of consequence. The total time scale is 16 weeks but there was some variation in the time intervals between trials. The arrows denote the commencement and termination of administration of kanamycin (400 mg kg^{-1} day^{-1}).

for 8 weeks. Throughout this time and for 4 weeks following the termination of drug administration DP was monitored daily (except Sat and Sun).

Results. The DP of 1 Lister hooded rat was stable throughout the period of testing which suggested that this animal was unaffected by the kanamycin dosage. With all the other rats there was a permanent impairment of DP and, with 2 exceptions (1 Wistar and 1 Lister hooded), the onset of this effect occurred during the last week of drug administration. The impairment progressed after termination of kanamycin dosage until, in all cases, DP was eventually extinguished completely (Figure 2). There was some individual variation in the time course of the progression of hearing impairment, as indicated by the time interval between the onset of impaired DP and its extinction. However, there was no distinguishable pattern in this between albino and pigmented animals. With the two exceptions, DP was first affected about 3 weeks before the end of drug administration and, in both cases, was extinguished before drug dosage was completed.

From Figure 2 it can be seen that there was a sudden reduction in the number of responses during tone, immediately following the start of kanamycin administration; this reduction, which occurred to some degree in all animals, subsequently recovered. It can be attributed to a transient effect of the drug on the animals' hunger drive[5].

Comment. This study revealed no difference between the susceptibility to the ototoxic effects of kanamycin of albino and pigmented rats. A great many laboratory animal studies have explored the effects of a variety of ototoxic drugs, but most authors have failed to report whether they used albino or pigmented animals, or both. However, both functional[6,7] and histopathological[8–10] changes have been observed in albino animals following the administration of ototoxic antibiotics. Consequently, there has never been any question that albino animals might be immune from ototoxicity by virtue of their having no melanin pigment. Furthermore, the present work suggests that albino animals are no less susceptible to ototoxicity than pigmented animals.

It now appears necessary to question the significance of the empirical observations that melanin pigment has a high affinity for known ototoxic drugs and causes them to accumulate at its sites of location in the body[3]. The principal site of location in the cochlea is the stria vascularis. Several workers[11–13] have observed strial lesions in guinea-pigs injected with ototoxic drugs and one study[3] related the damage to pigmentation since kanamycin was found to have caused serious damage in the stria of pigmented guinea-pigs but not in albino animals. The stria vascularis is involved in the production of endolymph, a fluid which is in contact with the receptor hair-cells. Thus damage to the stria vascularis could affect the composition of endolymph and the hair-cells would almost certainly be very sensitive to such disruption of their immediate environment. We have earlier commented[4] on the suspicion that hair-cell damage may oc-

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cur secondary to pathological changes in the stria vascularis, although a causal relationship has not been proven.

However, it is possible that injury to the stria vascularis, associated with melanin-induced drug accumulation there, could result in changes in endolymph composition and ultimate hair-cell damage. If this were the mechanism of action then, given the observed differences in strial lesions between albino and pigmented animals, differences would be expected in the degree of hair-cell damage and consequently in the degree of hearing impairment. This appears not to be the case.

It might therefore be interesting to discover whether the accumulation of ototoxic drugs in the strial melanin would be reflected in higher levels of the drugs in the endolymph of pigmented animals, where they could exert a direct effect on the hair-cells. If such differences in drug endolymph level were not observed it would argue for a more significant role of direct effect of the drugs on the hair-cells rather than one mediated through strial damage and would explain the failure to observe any differences in hearing impairment between albino and pigmented animals.

Pseudolymphoma of Skin Induced by Oriental Hornet (*Vespa orientalis*) Venom

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Summary. Intradermal injection of saline suspension of *Vespa orientalis* venom sac to 57 black mice caused a local nodule composed o flymphocytes, few histiocytes and plasma cells 10 to 12 days following the injection. This reaction simulates the pseudolymphoma reaction observed in humans following arthropode stings.

The histological lesions in arthropode stings have two distinct components which may or may not be combined. Changes in the epidermis manifested by pseudoepitheliomatous hyperplasia and dermal infiltration [1-3].The dermal inflammatory infiltration may consist either of necrotizing lesions with abundant eosinophilic leucocytes, or as a dense lymphocytic infiltration [1-3]. This dense lymphocytic infiltration can simulate lymphoma infiltrating the skin and the term 'pseudolymphoma' was used [1,2]. In an extensive study of the effect of oriental hornet (*Vespa orientalis*) venom on various organs, intradermal injection of oriental hornet venom was examined in various animals [4,5]. In guinea-pigs and rats, intradermal injection of the venom causes an acute inflammatory response with abundant eosinophils [4,5]. Injection of hornet venom into young black mice causes a dense local lymphocytic infiltration simulating lymphoma.

Twelve 10-day-old C 57 black mice were injected with a saline suspension of *Vespa orientalis* venom sac (150 mg/0.1 ml) intradermally in the back area. The animals were sacrificed at days 2, 4, 6, 10, 12 after injection and the

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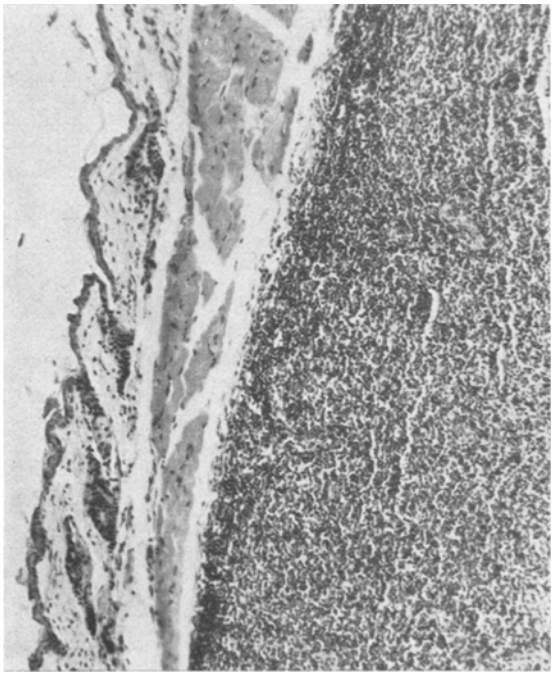

Fig. 1. Skin showing normal epidermis and upper dermis. A dense infiltration composed mainly of lymphocytes in the lower dermis. Hematoxylin-eosin stain. × 80.

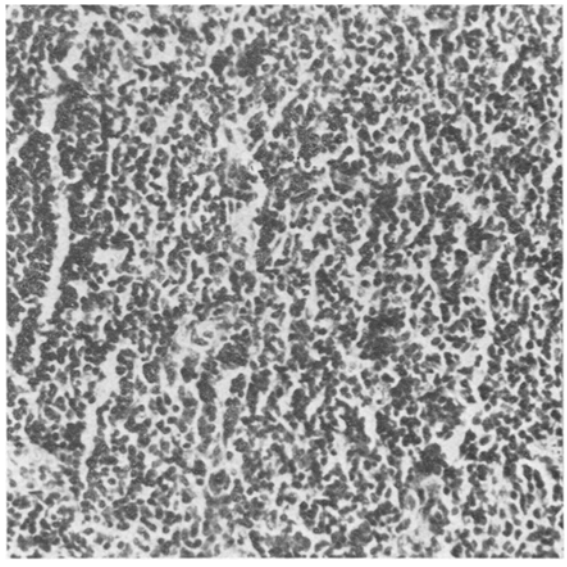

Fig. 2. Higher magnification of the dermal infiltration. Abundant small lymphocytes, few plasma cells and eosinophils. Hematoxylin-eosin. × 200.