been separated and isolated by means of chromatography in columns of 'Sephadex' gel. In a single experiment, mice were injected subcutaneously with this fraction (5·4 μg/g) dissolved in isotonic saline containing 0·01 normal formic acid. One day later, the mice were exposed to an X-ray dose of 850 r. Thirty days after irradiation 57 per cent of the mice so treated were still alive, while all the irradiated control mice injected with saline-formic acid had died.

At least three mechanisms may be invoked to account for the radioprotective effect of bee venom reported here for mice: (1) that it has a stressor-like action, thereby eliciting an "adaptation syndrome", assumed to increase radioresistance; (2) that it produces changes in the haemopoietic system, for example analogous to the effect of urethane[4] or of certain bacterial endotoxins[1]; (3) that it has antibacterial properties[13]. These mechanisms are now being investigated and further experiments are being carried out to separate, purify and identify the biologically active constituents of bee venom.

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MICROBIOLOGY

Tetramethyldipicrylamine—a New Antibacterial Agent

Moore, Meyer and Hudson recently reported on a new analytical reagent, tetramethyldipicrylamine (3,3′,5,5′-tetramethyl - 2,2′,4,4′,6,6′ - hexanitrodiphenylamine) and recommended it for further study in the analytical chemistry of the alkali metals[1]. A routine screening of this reagent by the National Cancer Institute, US National Institutes of Health, later showed its capacity to bring about a small but significant inhibition of tumour growth. A further check of its biological activity was then carried out, and is described here. The results indicate that this compound has antibacterial activity against Gram-positive organisms and several strains of sulphadiazine-resistant *Neisseria meningitidis*.

The sodium salt of tetramethyldipicrylamine was incorporated into trypticase soy broth and 1·5 per cent agar to give a final concentration of 10 and 50 μg/ml. medium. The following organisms were inhibited at the 10 μg/ml. concentration: *Staphylococcus aureus* (FDA-2098), *Staphylococcus epidermidis* (FDA-1200), *Streptococcus mitis*, *Streptococcus salivarius*, *Bacillus subtilis*, *Bacillus stereothermophilis* (NIII-7953). The following organisms were not inhibited at 50 μg/ml. concentration: *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella heidelberg*, *Serratia marcescens* (ATCC-13880), *Escherichia coli*, *Salmonella montevideo*.

Fourteen strains of *Neisseria meningitidis*, including both sulphadiazine resistant and sensitive organisms of group *B* and *C*, were tested. The assay was carried out on Mueller–Hinton agar as described by Frank, Wilcox and Finland[2]. The minimal inhibitory concentration ranged from 0·06 to 0·5 μg of the compound/ml. of medium.

Using the serial dilution assay method, *Bacillus subtilis* was inhibited by the compound at 1 μg/ml. and *Diplococcus pneumoniae* at 2–5 μg/ml. (Muir, R. D., personal communication).

A Gram-negative plant pathogenic bacterium *Erwinia* species and the fungi *Candida albicans* and *Fusarium* species were tested by an agar diffusion method. The compound was only slightly active against *Erwinia* species and had no activity against the fungi in this test.

No inhibition was found in tests using the nematode *Turbatrix aceti* or the protozoan *Trichomonas foetus* (Muir, R. D., personal communication).

These results show high activity *in vitro* against Gram-positive organisms and little or no activity against most Gram-negative organisms, fungi, nematodes and protozoa. It is interesting, however, to note that the compound shows high activity against the Gram-negative sulphadiazine-resistant meningococcal strains.

The compound, which is acidic and of low solubility, forms well defined salts. The sodium salt is readily soluble and was used for all our tests. The free acid showed no antibacterial activity.

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Ultrastructural Localization of Coxsackie B_4 Virus in Mouse Myocardium

COXSACKIE viral infections are known to cause myocardial lesions in various animal species[1]. The identification and localization of the Coxsackie viral particles in the heart have not been adequately demonstrated because of the absence of morphological criteria which will differentiate virus particles from glycogen granules and ribosomes. This communication describes the ultrastructural localization of Coxsackie B_4 virus particles in the mouse heart by a ferritin antibody technique.

A randomly selected litter of 5 day old suckling mice of *HaM/ICR* strain was inoculated intraperitoneally with 0·1 ml. of fluid containing 10^5 $TCID_{50}$ Coxsackie virus B_4 in monkey kidney cells; this strain was originally recovered by Kibrick and Benirschke from a 10 day old infant who died of encephalohepatomyocarditis[2]. The mice were killed 6 days after inoculation. Multiple 0·5 mm cubic blocks of tissue were cut from the myocardial wall and fixed in 4 per cent buffered glutaraldehyde for 20 min. The tissue blocks were washed in phosphate buffered normal saline, immediately immersed in a ferritin conjugated anti-Coxsackie virus B_4 rabbit serum for 45 min,

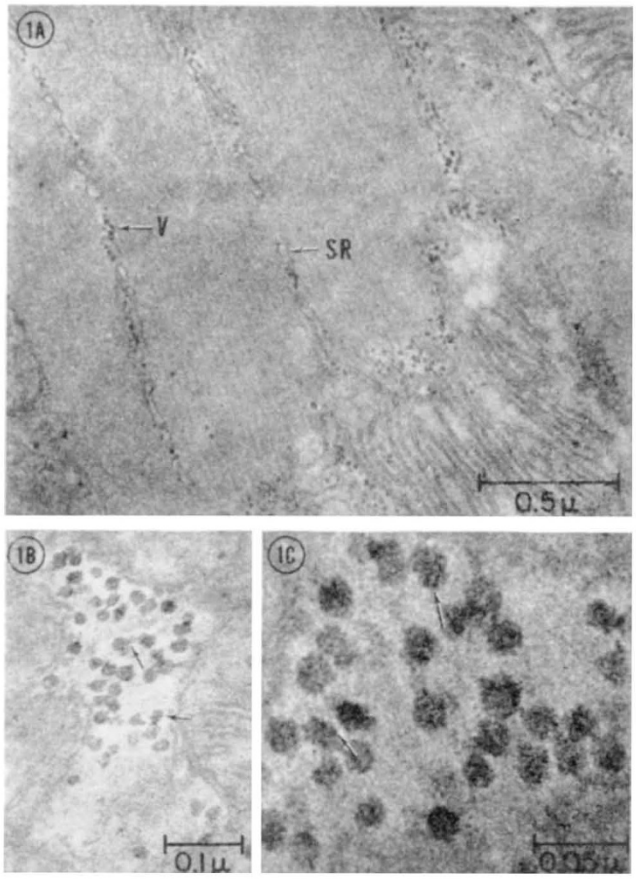

Fig. 1. An electron micrograph showing (*A*) the distribution of the Coxsackie B_4 virus particles (*V*) along the tubules of the sarcoplasmic reticulum (*SR*). (*B*) and (*C*) show viral particles at higher magnification, with the binding of ferritin molecules on the viral particle surface (arrows). Unstained.

washed in the buffer, fixed in osmium tetroxide, embedded in 'Maraglas' and examined with an electron microscope.

Conjugation of ferritin to previously prepared and purified hyperimmune anti-Coxsackie virus globulin (rabbit) was performed according to the method of Singer[4]. The ferritin acts as an electron dense "tag" which when coupled to antibody can be used to detect specific antigen–antibody reaction sites. Uninfected mice of the same age but from a different litter were used as controls. Control tissue was treated in the same way as that of experimental mice.

Virus particles were present only at certain foci within the infected hearts. In the infected muscle fibres the virus particles were oriented around tubules of the sarcoplasmic reticulum (Fig. 1*A*). No virus particles could be detected in the nuclei. The particles had a higher osmophilia than the surrounding matrix. Their average diameter was about 20 mμ. The viral particles were roughly hexagonal in profile and on their surface the small electron dense particles of ferritin could be easily identified (Fig. 1*B*, *C*). A few sparsely scattered free ferritin particles with no specific localization were also observed in the myocytes. In the control tissue, no such viral particles were noticed.

The early stages of Coxsackie infection produce few cytopathic features. Indeed, as with many other viral infections, synthesis of specific viral proteins apparently occurs simultaneously with normal cell protein and nucleic acid synthesis for varying periods of time[5]. The cells continue to function and show few alterations despite the presence of a latent infection. After 5–7 days definite pathological features appear in some cells and irreversible damage with necrosis becomes apparent[1,2]. The damage is focal, however, and some muscle cells appear entirely normal. It seems likely from other studies that many cells are initially infected and that the latent phase may exist for weeks or even years without evidence of overt cellular damage[6].

The distribution of the Coxsackie particles along the tubules of the sarcoplasmic reticulum seems significant. Since the inner membrane of the sarcolemma invaginates into the cell at the level of the *Z*-band and is in close approximation with the cavities of the sarcoplasmic reticulum, this may represent the primary route of virus entry into the sarcomere from the extracellular space.

The distribution of virus particles in the myocardium is evident from these observations. The ferritin labelling method is therefore a useful procedure for identification of viral infections of heart and other organs by routine electron microscopy.

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Mutation towards Dextran Sulphate Resistance in Type I Poliovirus

The use of type 1 poliovirus mutants resistant to dextran sulphate, as described by Takemoto and Liebhaber[1] and Takemoto and Kirschstein[2], has proved extremely promising for genetic investigations. We have therefore carried out some additional investigations of these mutants with the view of using them in recombination experiments.

The present communication reports preliminary observations on some characteristics of the mutation towards dextran resistance and the relationship between the *ds* marker and other genetic characters either belonging to the group displaying covariation with neurovirulence or independent of the latter.

Our experiments were performed with clonal derivatives of the attenuated type 1 LSc2ab and of the neurovirulent type 2 MEF_1 poliovirus strains. The behaviour of the viral populations towards dextran sulphate 2,000 was tested by the plaque method with dextran sulphate incorporated in the agar overlay at a concentration of 100 μg/ml., as previously described[3]. In some cases, in which the inhibitor gradient method[4] was applied, the dextran sulphate concentration in the gradient plate ranged from 0 to 200 μg/ml. The other markers were tested according to techniques described in previous papers[3,5]. The hydrochloric acid salt of 2-(α-hydroxybenzyl)-benzimidazole (HBB) used in some of our experiments was synthesized and kindly supplied by V. A. Blaszek from the Institute of Medicine and Pharmacy in Tg. Mureș (Romania). The inhibitory action of HBB was assayed according to the same techniques as that for guanidine[5].

When the parental L_1S type 1 attenuated poliovirus was exposed to a dextran sulphate gradient, the large plaques formed by ds^r particles were found to be scattered