PEA (10 to 50 mg kg^{-1}) alone (without MAOI pretreatment) induced depression preceded by a brief stimulation, whereas in mice pretreated with MAOIs (doses as described above), PEA induced amphetamine-like stimulation, 'popcorn' behaviour and, less frequently, aggressiveness. PEA (6 mg kg^{-1}) did not significantly alter the behaviour of mice not pretreated with MAOIs and, in those that were pretreated it induced hyperactivity and a weak and brief 'popcorn' effect. Moreover, the stimulant effects of Δ^9-THC and PEA are synergistic. In mice pretreated with nialamide (Table 2), the combination of Δ^9-THC (5 mg kg^{-1}) and PEA (6 mg kg^{-1}, 1 h after Δ^9-THC) induced marked hyperactivity, 'popcorn' behaviour and vicious fighting (defensive and aggressive postures, chasing, and biting to the point of drawing blood). A similar interaction between Δ^9-THC and PEA was obtained in mice treated with pargyline except that fighting was not observed.

These behavioural experiments suggest that the observed increase in the brain levels of PEA induced by Δ^9-THC are associated with an increase in free PEA available to receptor sites and that this is responsible for some of the stimulant effects of Δ^9-THC.

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Regeneration of the ventricular myocardium in amphibians

REGENERATIVE capacity of the myocardium in vertebrates is reported to be minimal[1-6]. Mauro has advanced the thesis that the absence of satellite cells in the cardiac muscle is responsible[7]. Most recently, Oberpriller and Oberpriller have reported mitosis in adult newt myocardial cells 16 d after minor trauma[8]. Here we report observations on the adult salamander which indicate that cardiac regeneration of great competency occurs in this animal.

The adult aquatic stage of *Triturus viridescens* was used, animals were stored in pond water in glass aquaria at room temperature and were fed chopped liver and beef twice weekly. Anaesthesia was obtained with 1:1,000 Tricaine (Ayerst) and the heart was approached through a midline ventral incision, splitting the sternum and incising the pericardial sac. Between 30% and 50% of the ventricular myocardium was removed with iridectomy scissors traversing across the ventricular cavity (Fig. 1) and the skin incision was closed with interrupted 4/0 silk sutures. The animals were immediately transferred to fresh pond water in finger bowls and after recovery, returned to glass aquaria. No antibiotics or other chemical agents were used. During recovery, skin capillaries were observed microscopically in the tail fin, using transmitted light. For histological examination, the heart was removed *in toto*, fixed in buffered formalin with subsequent routine processing in hematoxylin and eosin. For electron microscopy, the hearts were fixed in cold 4% phosphate buffered gluteraldehyde, post-fixed in osmium tetroxide and embedded in Epon. Thin sections showing silver-grey interference colours were stained with uranyl

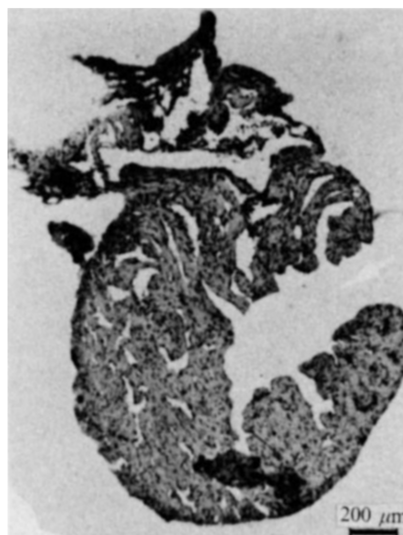

FIG. 1 Longitudinal sections of triturus heart showing extent of ventricular resection. Resection partially completed, entering into ventricular lumen from the right, in direction of the arrow. Excess portions of the auricles have been trimmed away after fixation.

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acetate and lead citrate and examined with an RCA EMU-2D electron microscope.

In an initial series of twenty animals, all survived the procedure for two weeks after which they were killed. Since then, several hundred animals have been used for various phases of the study with an overall mortality rate of 10%. Observation of the heart *in situ* by dissecting microscope immediately after ventricular resection revealed pronounced contracture of the myocardium at the resection site resulting in a decrease in the diameter of the opening into the ventricular lumen. Bleeding was noted to be initially brisk, decreasing rapidly and ceasing with clot formation at approximately 60 s.

Complete circulatory stasis occurred within 5 min of resection. Erythrocytes were always trapped in the observed capillaries and after a variable period of time (30–120 min), oscillatory movement of these cells could be seen, with no directional flow. Directional movement (in which pulsations as such were not visible) began generally at 90 to 120 min but once occurred as early as 1 h. Circulation was noticeably slower than normal and generally did not become normal until 3–5 h after resection. Twelve animals were followed using smears (Giemsa-Jenner stained) of the peripheral blood at 30 min intervals following resection. At 2 h after resection, immature erythrocytes first began to appear and reached a maximum 15%–20%) at 5 h.

Specimens for histological examination were taken at 5, 15, 30 and 60 min and 2, 3, 5 and 24 h after resection. Normal, non-operated specimens were processed at intervals to check on technique; no mitotic figures were observed in either the control or any of the experimental specimens. In the early experimental specimens, the major part of the clot was frequently lost in the process of removal and fixation of the heart. Many immature erythrocytes were visible, however, in the retained portions of the clot which were closest to the injured myocardium. After 30 min, the clot appeared to have differentiated into two zones, one closest to the injured myocardium which was composed of small basophilic cells with darkly staining nuclei and a peripheral zone of normal appearing erythrocytes with eosinophilic cytoplasm. After 3 h, the area of the resection was filled with a tightly packed mass of the basophilic cells (Fig. 2) and the original peripheral erythrocytes were undergoing degeneration and nucleolysis. Between 3 and 5 h, the mass of basophilic cells diminished and slightly basophilic myocardial fibres appeared, which were arranged in a more open 'lattice-like' fashion than the remainder of the myocardium. By 24 h, sections appeared completely normal and there was no evidence of injury other than, occasionally, a small mass of degenerating erythrocytes exterior to the myocardium proper.

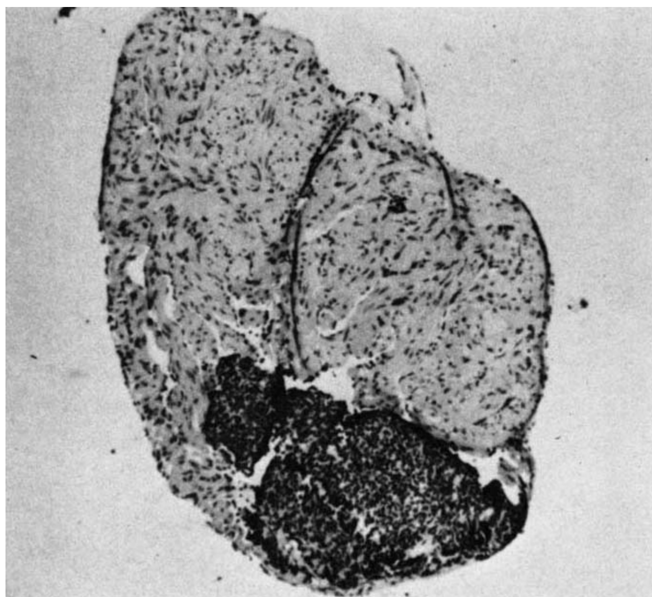

Fig. 2 Longitudinal section through ventricular myocardium 3 r after resection (magnification scale as Fig. 1). The mass of basophilic cells is clearly distinct, from the remainder of the myocardium and appears to be approximately the same in extent as the original resected portion.

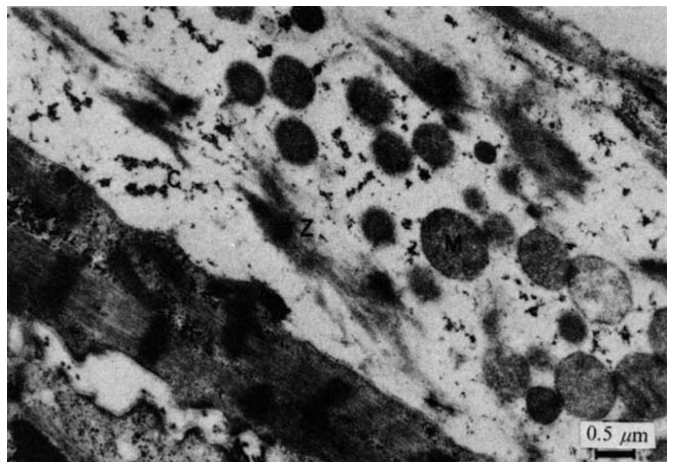

Fig. 3 Electron micrographs through site of ventricular resection two hours after resection; section through one of the cells bordering the defect, showing a region of active myofilament synthesis. Many polyribosomal complexes (C) are seen in close association with filamentous structures. z-band material and associated myofibrils (Z) are seen in the early stages of organisation. Mitochrondria (M) show densely packed cristae and an electron-dense matrix and the sarcoplasmic matrix has a plae embryonal appearance.

Specimens for electron microscopy were taken on the same time scale as for histological examination (except at 24 h). The early stages, 5 to 30 min, primarily showed the young forms of erythrocytes previously noted. The myocaridum at the edge of the defect demonstrated several signs of degeneration in the 30 min specimen. By 2 h, the cells closest to the myocardial edge showed active fibrogenesis (Fig. 3).

Unquestionably, these animals can survive resection of up to 50% of the ventricular myocardium, with restoration of normal circulatory dynamics within 5 h. It seems that this is accomplished by some active cellular process with restoration of the missing myocardium rather than simple approximation of the cut edges and subsequent 'sealing off'. The origin of the reparative cells and the mechanism used to restore the myocardial mass is, as yet, unclear. If this represents a true regenerative process, it is more rapid and competent than any previously reported.

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Thymine-labelled deoxyoligonucleotide involved in DNA chain growth in *Bacillus subtilis*

OKAZAKAI *et al.* showed that bacteria labelled with short pulses of radioactive thymidine incorporate radioactivity into small DNA fragments, the so-called Okazaki pieces[1]. These fragments have been observed in various bacterial, bacteriophage and eukaryotic systems[2,3]. Pulse-chase experiments demonstrated that the Okazaki pieces are precursors of longer DNA chains, indistinguishable in length from the bulk of the DNA. Thus, the idea has arisen that DNA synthesis proceeds discontinuously on at least one of the two growing DNA chains of a given replication fork; that is, individual short DNA fragments are synthesised by a 5′ → 3′ DNA polymerase and then joined to the growing chain by ligase[2].

However, Werner has reported that cultures of *E. coli* labelled with short pulses of ^{3}H-thymine incorporated radioactivity principally into large DNA; only a small fraction of the label was found in Okazaki pieces[4]. Furthermore, the fraction of the total label incorporated into Okazaki pieces increased rather than decreased with time of labelling during very short pulses. On the basis of these and other experiments, Werner concluded that DNA replication proceeds continuously without Okazaki pieces as intermediates[4]. Furthermore, he suggested that thymine and thymidine label separate precursor pools, thymine being used for replication and thymidine being incorporated primarily into Okazaki pieces involved in DNA repair[5].

Using a thymine-requiring strain of *Bacillus subtilis*[6], we decided to explore the possible differences between thymine (T) and thymidine (TdR) labelling of DNA. Cultures (80 ml) of *B. subtilis thy⁻ trp⁻* were grown in minimal medium at 25°C until late exponential phase (1.5×10^8 cells ml^{-1}), centrifuged and resuspended in 5 ml of fresh medium containing 5 μg ml^{-1} T. The concentrated cells were incubated at 25°C for 10 min and then labelled with short pulses of ^{3}H-T or ^{3}H-TdR. Pulses were terminated with −20°C acetone, the cells lysed and the lysates sedimented in alkaline sucrose gradients.

Figure 1 compares the labelling patterns of cells pulsed with ^{3}H-T against ^{3}H-TdR. In both cases, most labelled DNA sediments slowly with an S value of 30 or less. Very little radioactivity is found at a position in the gradient corresponding to 75S, the sedimentation coefficient of bulk DNA under our conditions. (The radioactivity in the final fraction of each gradient is due to unlysed cells.) Figure 2 shows the labelling patterns found with longer pulses of ^{3}H-T. Again, most labelled DNA sediments more slowly than bulk DNA. The S value increases gradually as the pulse time increases, in a manner similar to that reported by Okazaki *et al.* for ^{3}H-TdR labelling of *B. subtilis*[2]. The gradual transition shown in Fig. 2 is characteristic of *B. subtilis*; a significant number of intermediate size chains are observed, suggesting that joining of Okazaki pieces may be slower in *B. subtilis* than in *E. coli*[2].

Results similar to those seen in Figs 1 and 2 are also obtained by using unconcentrated cells, or by using a different solution to stop the pulse (10% pyridine-20 mM KCN − 1 mM EDTA at 0°C)[7]. In all cases thymine labels

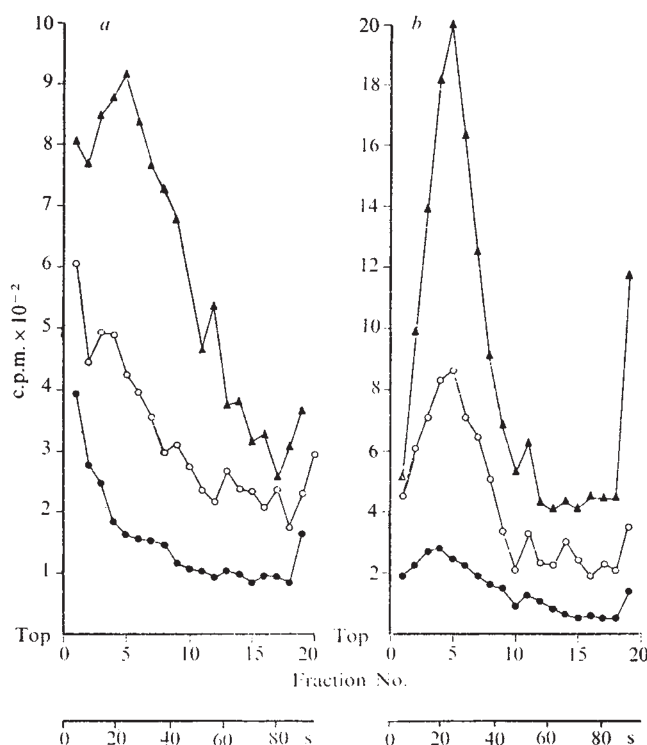

FIG. 1 Alkaline sucrose gradient sedimentation of pulse-labelled *B. subtilis* DNA. *B. subtilis thy⁻ trp⁻* was grown at 25°C in a medium containing 0.2% $(NH_4)_2SO_4$, 1.4% K_2HPO_4, 0.6% KH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 10^{-6} M $FeSO_4$ $7H_2O$, 10^{-7} M $MnCl_2 \cdot 4H_2O$, 0.2% glucose, 0.2% Na_3 citrate· $2H_2O$, 0.2% casamino acids, 40 μg ml^{-1} tryptophan, and 5 μg ml^{-1} thymine. The doubling time is 110 min in this medium. For a single pulse labelling experiment, 80 ml of cells was grown to late exponential phase (1.5×10^8 cells ml^{-1}), collected by centrifugation at 4°C, and resuspended in 5 ml of fresh complete medium. The concentrated cells were shaken gently for 10 min at 25°C, then stirred vigorously with a magnetic stirrer, and pulse labelled with either 0.6 ml ^{3}H-T (20 Ci mmol^{-1}, 1 mCi ml^{-1}) or 0.6 ml ^{3}H-TdR (6 Ci mmol^{-1}, 0.1 mCi ml^{-1}). The pulse was terminated by the rapid addition of 20 ml of −20° acetone. The cells were centrifuged at 4°C and then resuspended in 20 ml of a solution containing SSC (0.15 M NaCl, 0.015 M Na_3 citrate), 20mM KCN, 1mM EDTA. The cells were centrifuged again at 4°C and the cell pellet suspended in 0.15 ml of SSC containing 27% sucrose, 20 mM KCN, 10 mM EDTA, *p*H 8.2. A 25 μl sample of 40 mg ml^{-1} lysozyme was added and the sample incubated for 30 min at 37°C. Then 0.05 ml of 10% sarkosyl and 0.1 ml of 1 M NaOH − 0.1 M EDTA were added. After gentle shaking, the cells began to lyse; 0.7 ml of 0.3 M NaOH and 30 mM EDTA were added slowly. The entire lysate was layered on top of a linear 5% to 20% (w/v) alkaline sucrose gradient containing 0.3 M NaOH, 0.5 M NaCl, 10 mM EDTA. Sedimentation was carried out in a Beckman L2-65B ultracentrifuge with an SW27 rotor spun at 20,500 r.p.m. for 16 h at 4°C. The centrifuge tubes were fractionated from the top with an Auto Densi-Flow fractionator (Buchler Instruments) into 19 fractions of equal volume. Individual fractions were precipitated by addition of 0.3 ml 0.1 M Na pyrophosphate, 0.3 ml 3 mg ml^{-1} T, and 4 ml cold 15% TCA containing 0.50 mM Na pyrophosphate. The samples were kept at 0°C for 20 min, and the precipitates collected on nitrocellulose filters (Matheson-Higgins Co., 25 mm diameter, 0.45 μm pore size) which had previously been soaked in 50 mM Na pyrophosphate, 200 μg ml^{-1} T. The filters were rinsed with cold 3% TCA, dried, suspended in a toluene-scintillator solution, and counted in a liquid scintillation counter. The S scale was determined using a Φ29 DNA marker (S = 26). *a*, cells pulsed with ^{3}H-T; *b*, cells pulsed with ^{3}H-TdR. Pulse time: ●, 8s; ○, 15s; ▲, 30s.

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