whilst the low dose had no numerical effect, these cells were almost doubled by the high dose especially from 15 weeks.

Type I epithelium (figure 1, b) underwent no qualitative changes but cells became more numerous in longer-surviving, high-dose mice. If type I cells are derived from type II[5], augmentation of the former may merely reflect the situation with respect to the latter. Endothelial cells of irradiated mice in either group showed no quantitative or apparent qualitative abnormality.

Discussion. The observations point to type II epithelium as the prime sufferer from prolonged contact with plutonium emissions. Vacuolation of osmiophilic lamellar bodies has been noted after external application of X-rays[6] or ^{60}Co irradiation[7], but not to the same degree as in the present plutonium study. Such changes were not recorded in a study of the effects of $^{239}PuO_2$ inhalation[8]. Our ultrastructural evidence suggests a sequence of events, type II cells reacting by proliferation and probably by augmented secretory activity. Whether the surface properties of these lungs and their capacity for particle elimination are affected and whether the late phase of type II regeneration may progress to neoplasia remain to be determined.

The number and contents of alveolar macrophages rose notably in mice surviving the high-dose for longer periods, as did the population of interstitial cells, whose migration evidently serves to replace alveolar macrophages that disintegrate under their load or are carried proximally by ciliary activity. Despite the ingestion of or proximity to plutonium particles, macrophages retain their phagocytic capacity and are much less susceptible to the effects of irradiation than type II cells. Fibrosis was not a feature.

The time-dose relationship of the cellular events is epitomized in the table. Type II cells react to a wide variety of irritants and, although not necessarily specific, the features now described appear to be a particular consequence of irradiation especially by plutonium.

Plutonium induced changes in alveolar cells

	Quantitative		Qualitative	
	Low dose	High dose	Low dose	High dose
Type II epithelium	++	++	+→++	++→++++
Type I epithelium	nil	++	nil	nil
Alveolar macrophages	nil	++++	++	++→++++
Interstitial cells	nil	++++	nil	nil

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Effects of some oligopeptides, consisting of aromatic amino acids, on the excitability of an identifiable giant neurone of an African giant snail (*Achatina fulica* Férussac)

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Summary. L-Phe-L-Tyr and L-Lys-L-Phe-L-Tyr showed a marked inhibitory effect (not chloride-dependent) on the excitability of an identifiable giant neurone (the TAN) of Achatina fulica Férussac, while L-Tyr-L-phe, L-Tyr-L-Tyr, L-Phe-L-Phe, L-Lys-L-Phe and Z-L-Phe-L-Tyr (Z-: carbobenzoxy) had no effect.

In a previous paper[2], a marked inhibitory effect of a tripeptide (L-Lys-L-Phe-L-Tyr), produced as a fragment of physalaemin (a hypotensive endecapeptide from an Amphibian skin[3,4]), on the excitability of a giant neurone (the TAN, tonically autoactive neurone), identified in the suboesophageal ganglia of Achatina fulica Férussac, was reported. In the present study, the effect of dipeptides and tripeptides, structurally related to L-Lys-L-Phe-L-Tyr, was examined on the same neurone, to determine the essential structure of these substances to produce the inhibitory effect.

Material and methods. The experimental material used and the electrophysiological methods employed have been precisely described in previous papers[5–7]. The TAN (tonically autoactive neurone) of Achatina fulica is excited by 5-hydroxytryptamine and physalaemin and inhibited by dopamine, GABA and acetylcholine. Most dipeptides and tripeptides examined in the present study (table) were obtained commercially. In order to determine whether the inhibition caused by some peptide is dependent on chloride ions, the inhibitory peptide was tested in the chloride free condition. To remove the chloride ions from the extracellular fluid of the dissected ganglia, the isotonic acetate (chloride free) solution was continuously perfused around the ganglia for at least 15 min.

Results and discussion. Experimental results obtained are summarized in the table. L-Lys-L-Phe-L-Tyr and

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Effects of oligopeptides on the excitability of an identifiable giant neurone (the TAN, tonically autoactive neurone) of Achatina fulica Férussac (bath application in the physiological state)

	Substance	Effect on TAN	Examined concentration	
1	L-Lys-L-Phe-L-Tyr[a]	I	$5\times10^{-6}\sim10^{-5}$ kg/l*	($1.1\times10^{-5}\sim2.2\times10^{-5}$ M*)
2	L-Phe-L-Tyr[b]	I	$5\times10^{-6}\sim10^{-5}$ kg/l*	($1.5\times10^{-5}\sim3.0\times10^{-5}$ M*)
3	Z-L-Phe-L-Tyr[c]	(–)	10^{-4} kg/l	(2.2×10^{-4} M)
4	Z-L-Lys (Z)-L-Phe-L-Tyr[a]	I	2×10^{-5} kg/l	(2.6×10^{-5} M)
5	L-Tyr-L-Phe[d]	(–)	2×10^{-4} kg/l	(6.1×10^{-4} M)
6	L-Lys-L-Phe[a]	(–)	2×10^{-4} kg/l	(6.8×10^{-4} M)
7	L-Phe-L-Phe[d]	(–)	2×10^{-4} kg/l	(6.4×10^{-4} M)
8	L-Tyr-L-Tyr[d]	(–)	2×10^{-4} kg/l	(5.8×10^{-4} M)
9	L-Phe-L-Phe-L-Phe[d]	(–)	2×10^{-4} kg/l	(4.4×10^{-4} M)
10	L-Tyr-L-Tyr-L-Tyr[d]	(–)	2×10^{-4} kg/l	(3.9×10^{-4} M)
11	L-Phe-Gly[d]	(–)	2×10^{-4} kg/l	(9.0×10^{-4} M)
12	L-Tyr-Gly[d]	(–)	2×10^{-4} kg/l	(8.4×10^{-4} M)
13	Gly-L-Phe[d]	(–)	2×10^{-4} kg/l	(9.0×10^{-4} M)
14	Gly-L-Tyr[d]	(–)	2×10^{-4} kg/l	(8.4×10^{-4} M)

I, Inhibitory effect; (–), no effect. *Critical concentrations to produce the effect. Z-, Carbobenzoxy-; Z-L-Lys(Z)-, L-2,6-dicarbobenzoxyamino hexanoyl. [a]Donated by Dr A. Inoue of Daiichi Pharmaceutical; [b]product of Bachem Inc., California (USA); [c]product of Protein Research Foundation, Osaka; [d]product of Sigma Chemical (USA).

L-Phe-L-Tyr showed an inhibitory effect on the TAN excitability. The intensity of their effect is almost identical; critical concentrations which produce the effect (bath application) were about $5\times10^{-6}\sim10^{-5}$ kg/l ($1.1\times10^{-5}\sim2.2\times10^{-5}$ M for L-Lys-L-Phe-L-Tyr; $1.5\times10^{-5}\sim3.0\times10^{-5}$ M for L-Phe-L-Tyr). On the other hand, L-Tyr-L-Phe, L-Lys-L-Phe, L-Phe-L-Phe, L-Tyr-L-Tyr, etc. had no effect at 2×10^{-4} kg/l. While Z-L-Phe-L-Tyr (Z: carbobenzoxy) had no effect on the TAN at 10^{-4} kg/l, Z-L-Lys(Z)-L-Phe-L-Tyr (Z-L-Lys(Z)-: L-2,6 dicarbobenzoxyamino hexanoyl) (slightly soluble in the snail's physiological solution[8]) showed an inhibitory effect, the intensity of which was weaker than those of the 2 inhibitory peptides mentioned above. In figure 1, the intensity of the effect of these peptides is compared.

Figure 2A demonstrates that the microdrop application of L-Phe-L-Tyr of about 260 pg to the TAN surface almost immediately hyperpolarized the TAN neuromembrane and ceased the spike discharges in the physiological state. The biopotential changes caused by the dipeptide recovered completely several minutes after the application. The inhibitory effect of the dipeptide (microdrop application) was also demonstrated even in the isotonic acetate (chloride free) solution (figure 2B). The effect of L-Phe-L-Tyr may be due to hyperpolarizing directly the TAN neuromembrane, since the local (microdrop) application of the dipeptide showed the effect. The

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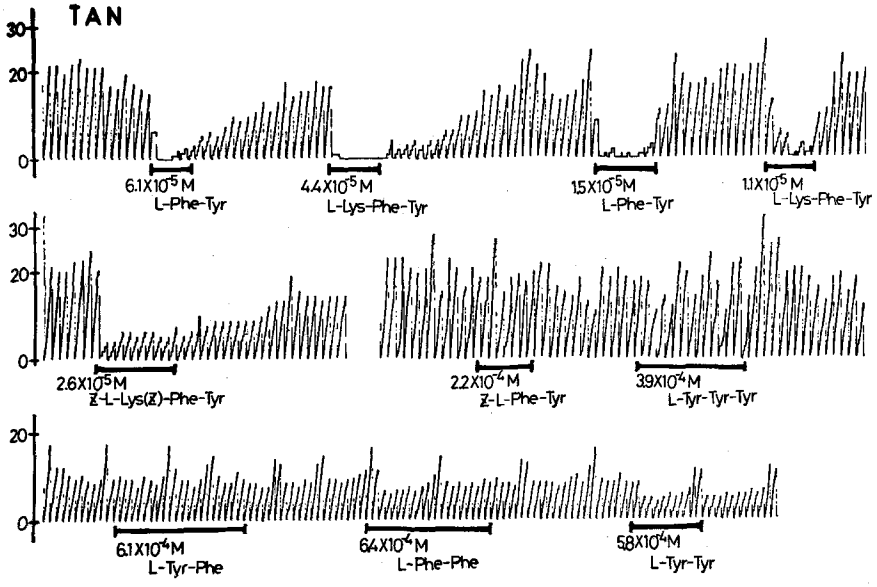

Fig. 1. Inhibitory effect of oligopeptides consisting of aromatic amino acids on the TAN (tonically autoactive neurone) excitability (bath application). 3 traces were recorded continuously. Ordinate, the number of spike discharges per min. Abscissa, the time course, each histogram is 1 min. 6.1×10^{-5} M (2×10^{-5} kg/l) L-Phe-L-Tyr, 4.4×10^{-5} M (2×10^{-5} kg/l) L-Lys-L-Phe-L-Tyr, 1.5×10^{-5} M (5×10^{-6} kg/l) L-Phe-L-Tyr, 1.1×10^{-5} M (5×10^{-6} kg/l) L-Lys-L-Phe-L-Tyr, 2.6×10^{-5} M (2×10^{-5} kg/l) Z-L-Lys (Z)-L-Phe-L-Tyr (Z-L-Lys (Z)-: L-2,6-dicarbobenzoxyamino hexanoyl), 2.2×10^{-4} M (10^{-4} kg/l) Z-L-Phe-L-Tyr (Z-: carbobenzoxy), 3.9×10^{-4} M (2×10^{-4} kg/l) L-Tyr-L-Tyr-L-Tyr, 6.1×10^{-4} M (2×10^{-4} kg/l) L-Tyr-L-Phe, 6.4×10^{-4} M (2×10^{-4} kg/l) L-Phe-L-Phe and 5.8×10^{-4} M (2×10^{-4} kg/l). L-Tyr-L-Tyr were applied respectively.

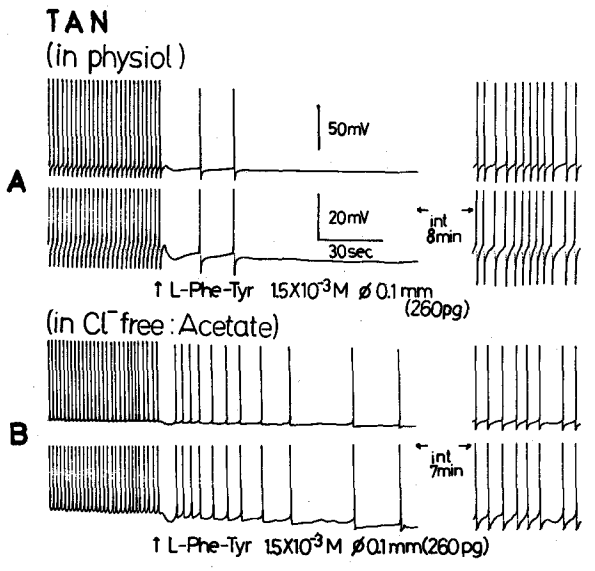

Fig. 2. Effect of L-Phe-L-Tyr on the TAN biopotential in both physiological state (A) and chloride-free medium (B) (microdrop application). The upper traces of A and B are full-spike recordings of the PON biopotential by a pen-writing galvanometer. The lower traces of A and B are high amplification recordings of the upper traces (spike peaks have been cut by an electronic voltage clipper). In both physiological state (A) and chloride-free medium (after the perfusion of the isotonic acetate [chloride free] solution for at least 15 min) (B), a microdrop (100 µm in diameter) of 1.5×10^{-3} M (5×10^{-4} kg/l) L-Phe-L-Tyr solution (the total amount of the dipeptide estimated to be about 260 pg) was applied on the TAN surface (arrow). Upper vertical bar, calibration for the upper traces (50 mV). Lower vertical bar, calibration for lower traces (20 mV). Horizontal bar, the time course (30 sec).

TAN inhibition caused by the dipeptide is not dependent on the permeability increase of the neuromembrane to chloride ions, since the presence of chloride ions in the medium is not necessary for the dipeptide to show the inhibitory effect.

It was previously reported[9] that each amino acid, of which the inhibitory peptides mentioned consist, had no effect on the TAN. Of the substances examined in the present study, therefore, L-Phe-L-Tyr is thought to be the essential structure for producing the effect. Although we can cite the pioneering works of Kakimoto et al.[10,11] on the presence of glutamyl dipeptides in the mammalian brain, yet, in general, the presence and the role of oligopeptides in the nervous tissue remains obscure. We propose the possibility that the inhibitory effect of L-Phe-L-Tyr on the TAN might be physiological, the dipeptide acting perhaps as a neurotransmitter or as a structurally analogous substance to an unknown transmitter. Further studies will have to be performed in order to clarify this area of research.

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Inhibitory effect of taurine on decrease in the inotropic action of ouabain at high concentrations in isolated atria

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Summary. In vitro, taurine was shown to inhibit the decrease in the inotropic effect of ouabain at large doses in the normal and also low K^+ medium in which this decrease in the inotropism of ouabain was facilitated. This inhibitory effect of taurine was, at least in part, due to the inhibition of the efflux of intracellular K^+ in the isolated heart.

A large quantity of taurine was found in mammalian heart[1–3], and yet little is known about its function in the heart[4]. It has been indicated that taurine acts as an antiarrhythmic in vagotomized dogs[5], rats[6] and guinea-pigs[7]. The antiarrhythmic effect was suggested to be, at least in part, due to prevention by taurine of the efflux of intracellular K^+ associated with some drug-induced arrhythmias[6,8]. Since taurine failed to influence Na^+, K^+-ATPase activity or an interaction between ouabain and Na^+, K^+-ATPase[6,9], this enzyme seemed not to be concerned in the antiarrhythmic action of taurine.

In isolated hearts, however, taurine, being positively inotropic in guinea-pig auricles in both normal and low Ca^{++} medium[10], potentiated positive inotropic effects of strophanthin-K[11] and ouabain[12], and inhibited a decrease of contractile force by Ca^{++}-free media[13] in these preparations. It was indicated that the potentiating effect of taurine on the positive inotropic effect of ouabain was, to some extent, related to an accumulation of intracellular Ca^{++} in the taurine-loaded heart[12].

Thus, the interest in these reports comes from the findings that taurine antagonized the cardiotoxic action of digitalis, but potentiated the positive inotropic action. In addition, any antagonisms between taurine and cardiac glycosides on isolated heart preparations have never been reported. The present author, therefore, attempted to demonstrate an inhibitory action of taurine on a decrease of the inotropic action in high concentrations of ouabain in isolated atria. Since reduction of serum K^+ levels increased the positive inotropic response to ouabain[14] and enhanced digitalis toxicity[15] and cardiac uptake of ouabain[14], it was also examined whether or not the inhibitory effect was influenced in a low K^+ medium.

Methods and materials. Detailed methods have been described elsewhere[7]. Briefly, left atrial strips, prepared from male guinea-pigs (250–350 g) were driven by electric