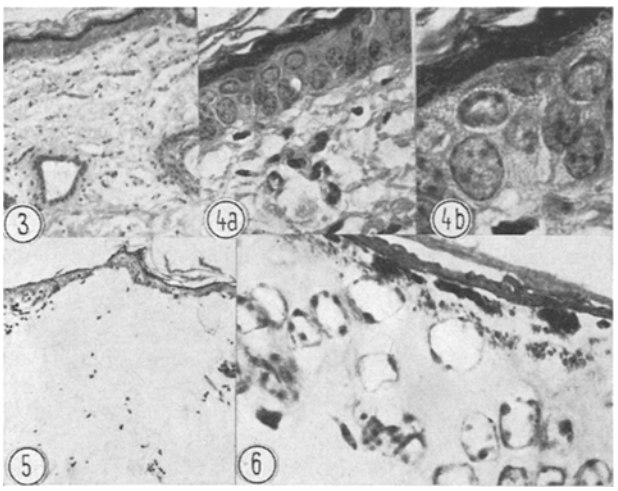

Figs. 3–6. Effect of DDT on the skin. 3, 4a and 4b showing the normal cells. 5 and 6, vacuolization and degeneration of cells (DDT-painted).

glutamine of brain. DDT when applied dermally to female guinea-pigs did, however, show certain interesting findings which included the depletion in the levels of different amino acids of the brain and kidney[12]. ROBERTS and TISHKOFF[14] reported a fall in the level of free amino acids of the tumors. These workers advocated that the pattern of free amino acids may be used as an appropriate tool in the identification of carcinomas from the non-malignant epidermis.

Histopathological findings also show that a constant application of DDT on the skin of guinea-pigs induces certain changes in the cell structure of the tissue. Thus, in comparison with the skin of control animals, DDT-painted skin showed the proliferation of hair follicles and increased degree of keratinization. Cytological observations also showed features of disruption and degeneration in the cells of the basal layer. Cells of the stratum malpighi carried vacuolated nuclei and 1–2 eosinophilic bodies indicating the early signs of damage (Figures 3–6). Depletion in the level of amino acids have a bearing on the structural changes of the cells observed under light microscope and electron microscope. While normal cells carry a highly developed endoplasmic reticulum, the same in the cells of the poisoned animal largely disappears and presents a vacuolated condition (details in COHEN et al.[15, 16]).

Zusammenfassung. Auf die Haut von Meerschweinchen appliziertes DDT bewirkt eine Verminderung im Aminosäuregehalt des Hautgewebes. Histopathologisch wurden Veränderungen im Bereich des Gewebes und der Zellen festgestellt.

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[1] 1,1,1-Trichloro-2,2-*bis*(P-chlorophenyl ethane) (P-P'DDT). P-P', isomer of 99.8% purity was generously supplied to us by J. R. Geigy & Co., Basle, and their help is gratefully acknowledged by the authors.
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Influence of Sodium on the Ability of Tyramine to Release Norepinephrine in Isolated Guinea-Pig Left Atrium[1]

It has been suggested that certain drugs are capable of enhancing the effects of norepinephrine and decreasing the effects of tyramine by blocking the uptake of both amines into adrenergic nerve endings (TRENDELENBURG[2]; FURCHGOTT et al.[3]; JOHNSON and KAHN[4]). More recently COMMARATO et al.[5] have presented evidence which supports the hypothesis that norepinephrine and tyramine are taken up by adrenergic nerve terminals through the same uptake system. If this were indeed the case one would expect that sodium deprivation, which has been shown to block the uptake of norepinephrine (IVERSEN and KRAVITZ[6]; GILLIS and PATON[7]; HORST et al.[8]; BOGDANSKY and BRODIE[9]), would also block the release of catecholamines induced by tyramine. The present experiments were undertaken in order to test this possibility.

Methods. Left atria from guinea-pigs were prepared and mounted, as previously described by FURCHGOTT et al.[3]. In each experiment one half of the atrium served as a control. The bathing solution was Krebs bicarbonate containing 10 m*M* glucose and 10^{-5} g/ml of ethylenediaminetetraacetic acid (EDTA). A mixture of 95% O_2 and 5% CO_2 was bubbled through the bathing solution. All preparations were electrically driven at a frequency of 30 beats/min. Atria were attached to force-dispacement transducers (Grass, Model FT.03) and mechanical activity was recorded by means of a Grass Polygraph. When low-sodium solution was used, the osmotic pressure of the solution was maintained with equivalent amounts of sucrose.

The catecholamines analysis was performed according to the method of ANTON and SAYRE[10]. Results are expressed as µg of norepinephrine base per g of tissue. All values reported in this paper are corrected for the degree of recovery.

The doses of drugs used (Iproniazid phosphate, norepinephrine bitartrate and tyramine hydrochloride) are expressed in terms of g of salts per ml of medium in the muscle chamber.

Table I. Release of norepinephrine (NE) by tyramine on isolated guineapig left atrium

N[a]	Exposure to tyramine (g/ml)	NE (μg/g) in tissue 10 min after washout of tyramine[b]	Difference
10	none	4.249 ± 0.289	0.625
	10^{-5} (for 10 min)	3.624 ± 0.294	($P < 0.02$)

[a] Number of experiments. [b] Mean ± standard deviation.

norepinephrine in the cytoplasm could explain the increased release of norepinephrine by tyramine.

The results of the present experiments also show that the norepinephrine releasing ability of tyramine decreases when the preparations were suspended in a low-sodium medium. Moreover, the norepinephrine content of both low-sodium tyramine treated and low-sodium control preparations is not significantly different, even though in both cases it is lower than that found in control, non-tyramine-treated preparations washed with regular Krebs.

These results could be interpreted in two possible ways. First, as meaning that sodium is required for the uptake

Table II. Effect of a sodium-deficient medium (25 m*M* Na) on the catecholamine content and on the release of norepinephrine (NE) by tyramine, in guinea-pig left atria pretreated with Iproniazid (5×10^{-4}, for 30 min) and incubated with NE

N[a]	State of MAO	NE present during incubation (g/ml)	Washout after NE incubation	Tyramine (g/ml) added 15 min after NE incubation	NE (μg/g) in tissue 45 min after washout of NE[b]	Difference
15	Inhibited	10^{-5} (for 30 min)	Normal Krebs	10^{-5} (for 10 min)	7.761 ± 0.735	2.436
	Inhibited	10^{-5} (for 30 min)	Normal Krebs	none	10.197 ± 0.590	($P < 0.01$)
11	Inhibited	10^{-5} (for 30 min)	Low-Na-Krebs	10^{-5} (for 10 min)	7.744 ± 0.680	0.536
	Inhibited	10^{-5} (for 30 min)	Low-Na-Krebs	none	8.280 ± 0.651	($P > 0.3$)
6	Inhibited	10^{-5} (for 30 min)	Low-Na-Krebs	none	7.881 ± 0.642	1.584
	Inhibited	10^{-5} (for 30 min)	Normal Krebs	none	9.465 ± 0.783	($P < 0.01$)

[a] Number of experiments. [b] Mean ± standard deviation.

Statistical significance of the difference between means was determined by the *t*-test for paired data.

Results. Preliminary experiments showed that exposure of one half of an atrium to tyramine (10^{-5} for 10 min) causes a small but significant release of norepinephrine (Table I). When the preparations are first treated with a monoamine oxidase inhibitor (Iproniazid, 5×10^{-4} for 30 min) and then incubated with norepinephrine (10^{-5} for 30 min), the releasing ability of tyramine greatly increases (Table II). Therefore all experiments reported in this paper were performed in monoamine oxidase inhibited preparations incubated with norepinephrine.

In a group of experiments, at the end of norepinephrine incubation both halves were washed with low-sodium Krebs (25 m*M* Na) and 15–20 min later one preparation was exposed to tyramine (10^{-5} for 10 min). As shown in Table II, no significant difference was found between the norepinephrine content of tyramine-treated and control preparations; however, in both cases the norepinephrine content was lower than that found in control, non-tyramine-treated atria, washed with regular Krebs.

In another set of experiments, at the end of norepinephrine incubation one half was washed with low-sodium Krebs and the other with regular Krebs. As shown in Table II, the norepinephrine content was significantly lower in the preparation washed with low-sodium Krebs as compared with the control washed with regular Krebs.

Discussion. The norepinephrine releasing ability of tyramine on isolated guinea-pig left atrium greatly increases when the preparations are pretreated with a monoamine oxidase inhibitor and subsequently incubated with norepinephrine. In a previous paper FURCHGOTT and SÁNCHEZ-GARCÍA[11] showed that in normal monoamine oxidase inhibited atrial preparations the retention of norepinephrine greatly increases; they suggested that this increase probably represents both the free and bound norepinephrine effectively retained in the cytoplasm because of protection from intraneuronal deamination. In our experimental situation, the protection of tyramine from deamination and the presence of free and loosely bound of tyramine by adrenergic nerve terminals and therefore for the subsequent release of norepinephrine. A second possibility is that tyramine and the sodium-deficient medium could release norepinephrine from a common pool. Which one of these two possibilities is correct cannot be decided at present.

Resumen. El contenido de catecolaminas de la auricula de cobayos tratada con Iproniazida e incubada con norepinefrina disminuye significativamente cuando se suspenden en un medio deficiente en sodio (25 m*M*). En estas condiciones el efecto liberador de norepinephrine inducido por tiramina desaparece.

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