calcium concentration of the perfusing medium was lowered to 0.22 m*M*. Apparently a minimum calcium concentration gradient across the medullary cell membrane must exist before acetylcholine-induced catecholamine release can occur.

Zusammenfassung. Nachweis an der isolierten perfundierten Rindernebenniere (5 ml/min), dass die Acetylcholin- oder Kalium-Stimulation der Catecholaminausschüttung bei fehlendem Calcium gleichmässig verhindert war. Ferner wurde die geringste Calciumkonzentration für den Ausschüttungsreiz bestimmt.

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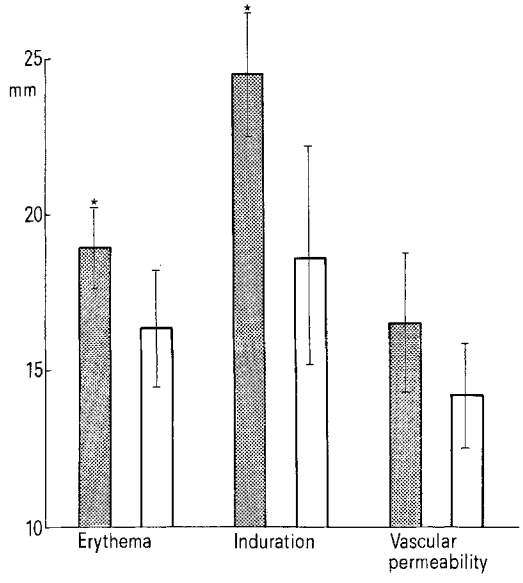
The Effect of N-(2-hydroxyethyl)-Palmitamide on Delayed Hypersensitivity in Guinea-Pig

It has been shown that N-(2-hydroxyethyl)-palmitamide (PEA) can decrease the intensity of several inflammatory and immunological processes in experimental animals[1–3]. Recently the interest on biological properties of PEA has been revived because of its capacity to increase nonspecific tolerance to several bacterial toxins[4]. In this communication the influence of PEA on some manifestation of tuberculine hypersensitivity in guinea pigs is reported.



Erythema, induration and vascular permeability of the skin tests in tuberculine hypersensitivity in guinea pigs. ▦, N-(2-hydroxyethyl)-palmitamide pretreated animals; □, controls. The values of induration are expressed as 10-fold magnification of skin double thickness measured by 'Schnelltaster' System Kröplin. The transverse diameter of erythema was measured with a millimeter ruler. Each group consists of 7 animals (mean ± limits of confidence). * $P < 0.05$ compared to controls.

Material and methods. Random bred male albino guinea-pigs weighing 250–350 g were used. Animals were fed with 50 mg of PEA per kg body wt. and day by stomach tube. The animals received PEA 13 times prior to sensitization, the last dose of PEA was administered on the day of sensitization. The animals were injected with 1 ml of Freund's complete adjuvant per animal into 4 foot pads and the neck. 3 weeks later the skin test, vascular permeability test and inhibition of migration of macrophages were performed. Skin tests were performed on shaven skin of dorsal flank of animals. 10 μg of PPD in 0.1 ml of saline was administered intradermally, while controls were given saline only. The vascular permeability tests were performed by i.v. injection of Evans blue in saline and measurements evaluated after 30 min and 24 h respectively as described previously[5].

The test of inhibition of migration of macrophages was performed on guinea pig spleen explants by a method described elsewhere[6].

Results. We have found that animals treated with PEA prior to sensitization displayed significant increase in the erythema intensity, diameter and induration values 24 h after PPD administration as compared to the controls (Figure). These results were in good accordance with values obtained by measuring vascular permeability. In the 30 min interval, the blueing displayed-as expected-minor values in both control and pretreated animals. On the other hand, in 24 h interval the PEA pretreated animals showed marked increase in diameter as compared to controls (Figure).

When spleen fragments from hypersensitive animals were tested on inhibition of macrophage migration in

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Mean values of cytotoxic indices (CI) from controls and N-(2-hydroxyethyl)-palmitamide pretreated animals

N-(2-hydroxyethyl)-palmitamide pretreated group	Individual animals 0.26 0.23 0.29 0.25 0.24	Mean ± limits of confidence 0.25 ± 0.026
Control group	0.65 0.38 0.25 0.26 0.40	0.39 ± 0.179

Each value of individual animal represents an average plotted as a mean of 9 spleen fragments.

tissue culture a decrease in cytotoxic indices (i.e. greater inhibition) in PEA pretreated animals has been found as compared to controls (Table).

Discussion. In the previous experiment when PEA was administered 6 weeks starting on the day of sensitization, the erythema of the tuberculine reaction in guinea-pigs was significantly decreased[3]. In our present experiment, the scheme of administration differed from the previous one. PEA was repeatedly administered prior to sensitization. The number of evaluated criteria was increased, as the skin test, its induration and inhibition of migration of macrophages are considered as the most significant features of delayed hypersensitivity. In this experimental design, PEA, contrary to the previous study, increased the erythema and induration of tuberculine reaction. There is also a satisfactory correlation between these in vivo tests and the test of inhibition of macrophage migration where the significant decrease of cell migration from explants was noted. From preliminary results not yet published PEA does not change the ability of the migration inhibitory factor (MIF) production by lymphoid cells in vitro. Our results suggest that PEA could play a modifying role in the course of development of certain immunological processes, and as the mechanism of action has not been clarified yet, further analytical studies are needed.

Zusammenfassung. Die Wirkung von N-(2-hydroxyethyl)palmitamid auf den Verlauf der Überempfindlichkeit des Spättypes in Meerschweinchen wurde verfolgt und festgestellt, dass die Verabreichung dieses Stoffes vor der Sensibilisierung eine Stimulation der Hypersensitivität bewirkt.

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Glutamate and GABA Levels and Glutamate Decarboxylase Activity in Brain Regions of Rats after Prolonged Treatment with Alkali Cations

Several reports have indicated the antagonistic effects produced by lithium and rubidium when administered to animals. Monkeys given chronic RbCl treatment showed changes in their behavior and EEG pattern opposite to those produced by LiCl[1]. Contrasting effects of lithium and rubidium on catecholamine metabolism were observed in rat brain[2]. Prolonged rubidium treatment resulted in excitation, aggression and fighting behavior in animals[1,3,4], whereas lithium reduced aggressive behavior and hyperactivity in animals[5–8]. It has, therefore, been suggested that rubidium might have therapeutic applications in affective disorders, particularly in depressions[9], and initial treatment has shown remarkable therapeutic response in depressed patients[10]. We have recently reported that repeated administration of low non-toxic doses of LiCl to rats produced differential changes in glutamate and γ-aminobutyric acid (GABA) levels in certain brain regions[11,12]. These observations, as well as the association of glutamate and GABA with the state of brain activity[13], led us to extend our previous work further[12], and to test the effct of other alkali monovalent cations, e.g. Na^+, Rb^+ and Cs^+, on the content of glutamate and GABA in several brain regions as well as on the enzyme glutamic acid decarboxylase (GAD) which forms GABA from glutamic acid.

Experimental. Male rats, 80–100 g from the Biodynamics Department Colony derived from Wistar stock, which had free access to Purina Laboratory Chow and water, were used. The treatment consisted of i.p. injections of 2 meq/kg of 1 *M* solutions of NaCl, RbCl or CsCl twice daily for 4 consecutive days. The last injection was administered 1 h before decapitation. The brain was removed without delay and different parts were dissected as described previous-

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Table I. Glutamate and GABA levels in brain regions of rats after prolonged treatment with alkali cations

Treatment	Glutamate (μmole/g fresh wt.) Cortex	Amygdala	Hypothalamus	Brain Stem	GABA (μmole/g fresh wt.) Cortex	Amygdala	Hypothalamus	Brain Stem
NaCl	13.6 ± 0.2 (7)	13.7 ± 0.4 (7)	11.0 ± 0.4 (7)	7.6 ± 0.3 (6)	2.1 ± 0.1 (7)	2.7 ± 0.2 (7)	4.3 ± 0.2 (7)	2.2 ± 0.1 (6)
RbCl	14.8 ± 0.4 (7)[b]	15.0 ± 0.4 (7)[b]	11.5 ± 0.4 (8)	8.3 ± 0.6 (6)	2.6 ± 0.2 (8)[a]	3.4 ± 0.2 (8)[a]	6.0 ± 0.3 (8)[c]	2.5 ± 0.3 (5)
CsCl	14.9 ± 0.4 (7)[b]	14.6 ± 0.3 (7)[a]	11.4 ± 0.3 (6)	8.0 ± 0.2 (6)	2.4 ± 0.2 (7)	3.0 ± 0.3 (6)	4.9 ± 0.3 (6)	2.5 ± 0.2 (5)

Each treatment (2 meq/kg) was given i.p. twice daily for 4 days. The last injection 1 h before sacrificing the rats. Means ± S.E. Number of samples in parentheses; each sample is composed of tissue pooled from 3 rats. [a] Difference from control rats (NaCl treated; see text) significant $P < 0.05$ (Student's t-test); [b] $P < 0.01$; [c] $P < 0.001$.