Table I. Effect of cadmium, lead and cadmium-lead on embryonic development in the hamster

	No. of mothers	Total No. of embryos	Normal	Total embryos resorbed	malformed
Cd (2 mg/kg)	7	76	43	21 (27%)	32 (42%)
Cd (2 mg/kg) + Pb (25 mg/kg)	9	122	30	40 (32%)	52 (42%)
Cd (2 mg/kg) + Pb (50 mg/kg)	8	86	0	40 (46%)	46 (54%)
Pb (50 mg/kg)	8	98	14	37 (38%)	47 (48%)
Controls (distilled water)	10	131	121	10 (7%)	0 (0%)

Table II. Malformations resulting from cadmium, lead and cadmium-lead in the hamster

	Exencephaly	Microophthalmia	Cleft lip/palate	Umbilical hernia	Tail
Cd (2 mg/kg)	13	5	19	0	0
Cd (2 mg/kg) + Pb (25 mg/kg)	20	7	8	10	30
Cd (2 mg/kg) + Pb (50 mg/kg)	9	1	0	3	65 (12[a])
Pb (50 mg/kg)	0	0	0	0	40

[a] Sympodia.

important roles in embryonic differentiation. Thus it is possible that under these experimental conditions cadmium and lead interact additively on certain enzyme systems in the case of the tail bud malformation, and that lead blocks the effect of cadmium on the differentiating visceral arch system preventing the facial abnormalities.

Further investigations on the permeability of the mammalian placenta to heavy metals and their localization in specific differentiating embryonic tissues are necessary to identify the exact mechanisms of these site-specific malformations as well as the complex interaction of these teratogenic agents[6].

Zusammenfassung. Bleisalze verursachten fötale Missbildungen der Kaudalregion, wenn sie trächtigen Goldhamstern am 8. Tage injiziert wurden. Kadmiumsalze hingegen verursachten hauptsächlich Gesichts-, Augen- und Gehirndefekte. Die hier beschriebenen Befunde zeigen, dass die Kombination dieser beiden Teratogene zu wesentlich reduzierten Gesichtsmissbildungen, aber erheblich potenzierten Kaudalskelettmissbildungen (Sympus) führt.

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Reversible Necrosis at the End-Plate Region in Striated Muscles of the Rat Poisoned with Cholinesterase Inhibitors

During a systematic study of the processes involved in spontaneous recovery of the rat after severe poisoning with cholinesterase inhibitors, morphological changes in the striated muscles were observed which hitherto remained unnoticed.

Materials and methods. Albino rats of 180–220 g were i.v. injected with a just sublethal dose of either DFP, tabun or paraoxon. The doses used were 1200, 110 and 450 µg/kg, respectively. At suitable intervals after the injection, animals were sacrificed and the diaphragm, the intercostal, the psoas, the gastrocnemius and the soleus muscles were examined histologically. 7 µ thick sections were stained with hematoxilin-eosin. If the sciatic nerve had to be stimulated, the rats were atropinized (50 mg/kg, i.p.) and kept under barbital anaesthesia. If also D-tubocurarine had to be given, the animals were kept alive with artificial respiration.

Results and discussion. About 2 h after the injection of the anticholinesterase localized eosinophilia, swelling of the sarcoplasm and loss of striations is seen in several muscle fibres. In an affected fibre a distinct demarcation exists between the abnormal and the normal parts. After 4–6 h segmented leucocytes appear, the sarcolemnal nuclei become pycnotic and the sarcoplasm breaks up in floes (Figure 1a). Approximately 12 h after the injection, a complete but localized necrosis has developed in the affected fibres. Subsequently histiocytes enter the necrotic parts and phagocytosis starts (Figure 1b). After 2–3 days the remnants of the necrotic sarcoplasm are completely removed and mesenchymal cells proliferate in the apparently empty tubes. Fibrils with striations appear 3–4 days after the anticholinesterase injection. After 10 days the previously necrotic parts are still recognizable as basophilic segments rich in nuclei, but the fibre unity is restored (Figure 1c).

The localized fibre necrosis was the more extensive the greater the activity of the muscle had been, the diaphragm showing more necrosis than the gastrocnemius and psoas muscles. Unilateral section of the phrenic nerve in 6 rats, before the injection of the poison, prevented the necrosis on that side. Unilateral stimulation of the sciatic nerve with one stimulus per 5 sec during 6 h in 5 anaesthetized, DFP-treated rats produced extensive necrosis on that side only.

Strikingly, the anticholinesterase soman, which does not attack the junctional transmission in the rat at a just sublethal dose, produced no necrosis.

Rats treated with the cholinesterase reactivating oxime pralidoxime (P2S), within 2 h after DFP poisoning, showed no necrosis. If the oxime injection was postponed, the necrosis developed as usual.

Direct observation of dissected, living diaphragms under the low-power microscope showed that muscles with necrosis have dark areas in the middle part of the

fibres, where the end-plates are situated (Figure 2). This phenomenon is first seen 4 h after the injection of the enzyme inhibitor, is fully developed after 10–20 h, and disappears on the fourth to fifth day.

Histological examination of sections parallel to the fibre direction confirmed that the areas of necrosis are mostly located in the middle of the fibres. Staining with goldchloride produced badly stained end-plates in the necrotic parts, which made them very difficult to find.

It was concluded that the necrosis is the result of the abnormal functioning of the myoneural junction when the cholinesterase activity is reduced. That this function was actually abnormal was demonstrated by studying the neuromuscular transmission in isolated diaphragms dissected from the poisoned rats. These muscles were tested with trains of indirect stimuli at 25, 50, 100 and 200/sec. A normal diaphragm is capable of sustaining a 10-sec tetanic contraction at all 4 frequencies. In the poisoned rat, 2–4 h after the injection of the standard dose of DFP, the isolated diaphragm has almost completely lost the ability to sustain a contraction at 200 stimuli/sec and the performance at 100/sec is subnormal, indicating that more than 85% of the cholinesterase is inhibited[1].

In the active muscles of the poisoned rat, the abnormally high level of acetylcholine (ACh) at the end-plate

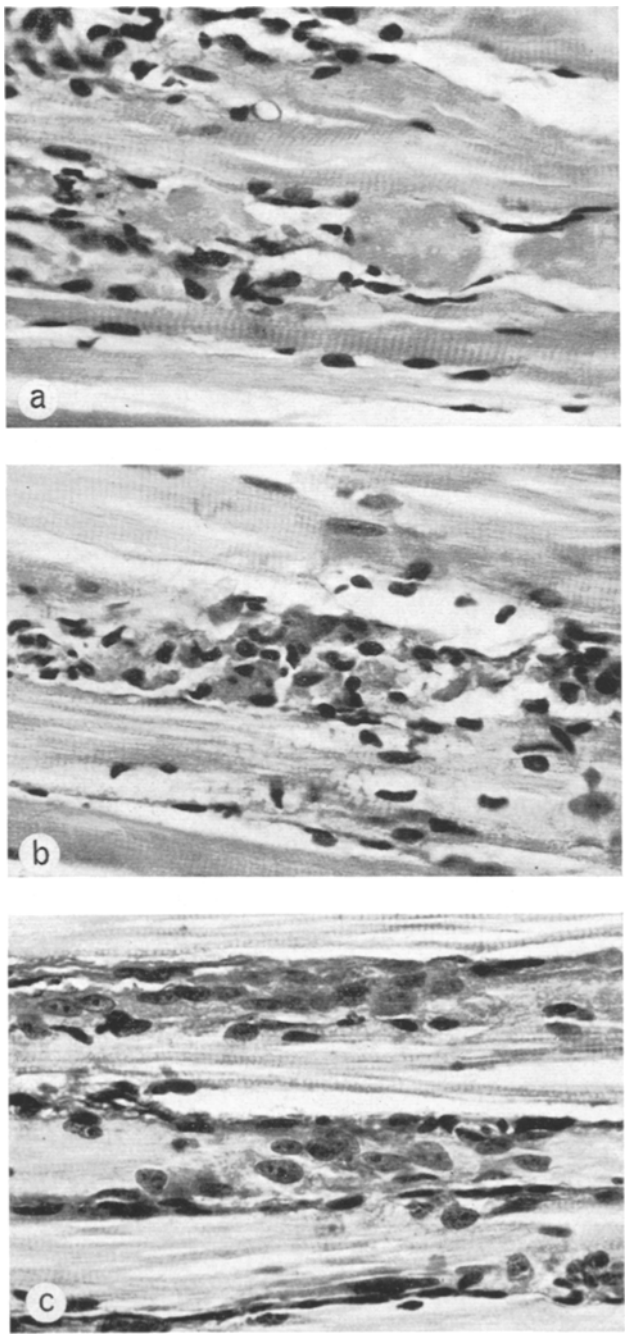

Fig. 1. Development of focal muscle fibre necrosis in diaphragm muscle. Hematoxilin – eosin staining. × 400. (a) 6 h after DFP injection, (b) after 24 h, (c) after 10 days.

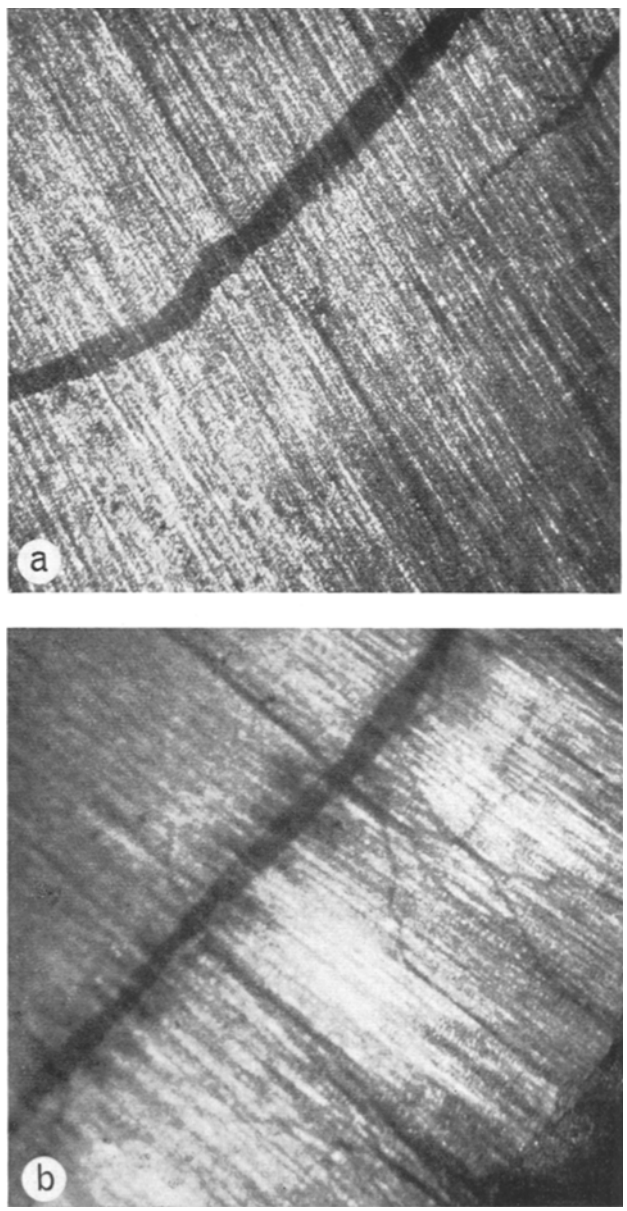

Fig. 2. Low-power microphotograph of end-plate area of living rat diaphragm, intramuscular branch of motor nerve visible. (a) normal muscle, (b) 8 h after DFP injection.

creates a long-lasting depolarization[2] resulting from a long-lasting change in permeability of the junctional membrane. If this prolonged permeability change is the cause of the necrosis, it should be possible to prevent this by protecting the junctional membrane against the ACh. In 5 DFP-poisoned rats necrosis of the indirectly stimulated gastrocnemius and soleus muscles could indeed be prevented by D-tubocurarine given in a dose of 8 mg/kg, i.p., every 2 h, during the 6 h of stimulation. However, since the muscles in these experiments were inactivated by the curare block, the inactivity of the muscle, as such, might have prevented the necrosis. This was shown not to be the case: if the curare regime was reduced to 0.75 mg/kg each hour, the muscles were still completely paralysed but marked necrosis occurred in the stimulated muscles in 3 out of 4 animals. In these rats the junctional membrane was still too insensitive to ACh for transmission to occur but obviously sensitive enough to allow necrosis to develop.

The results of the experiments show that necrosis occurs in the region of the motor end-plates in fibres of active striated muscles in rats poisoned with cholinesterase inhibitors, probably as a consequence of the presence of abnormal amounts of ACh.

Résumé. Après l'injection d'un toxique anticholinestérasique à dose presque léthale, une nécrose focale des fibres musculaires striées du rat se développe en 24 h. 10 jours après l'injection, la régénération est presque totale. Les parties nécrotiques se trouvent dans les régions des plaques motrices. Les résultats obtenus suggèrent que l'action dépolarisante de l'acétylcholine au niveau des plaques motrices des muscles actifs, augmentée et prolongée par l'intoxication, est responsable de la nécrose.

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Ineffectiveness of Carboxypeptidase B in Inhibiting the Pressor Effect of Incubated Human Plasma

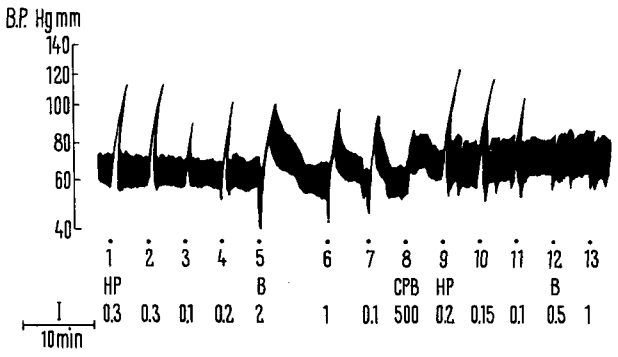
The i.v. injection of 0.1 ml human plasma incubated for several hours at 38 °C into a hypotensive rat produces an increase in the blood pressure equivalent to 5–10 ng of angiotensin II[1,2]. Contrarywise a hypotensive reaction is observed when the same plasma is injected into a cat. These pharmacological features are common to bradykinin[3] and kallidin[4] and suggest that these polypeptides could participate in the action of the incubated plasma.

The demonstration[5] that the carboxypeptidase B in vivo can block the effect of kinins and kinogenases (kallikreins), gives the possibility of investigating whether a liberation of a plasmakinin is involved in the pressor effect of the incubated plasma.

Materials and methods. Nephrectomized rats, operated upon 12–16 h before and anaesthetized with an i.p. injection of dialylbarbituric acid urethane solution (Dial, CIBA) in a dose of 0.1 ml/100 g body weight were used. The blood pressure was measured by introducing a cannula into a carotid artery connected to a membrane manometer (Hürtle type). Heparin was used as anticoagulant.

The incubated plasma and the other substances were injected into the femoral vein through a polyethylene tube. Valyl-5-amide angiotensin II (CIBA) and bradykinin (Sandoz) dissolved in 0.1 ml of NaCl 0.9% were used as standards. Plasma was obtained from healthy adult persons. Enough heparin to get a final concentration of 0.5 or 2 IU/ml, when mixed with the blood, was introduced in sterilized, siliconized bottle, before bleeding. After centrifugation the plasma was transferred to another sterilized siliconized bottle by aspiration, and placed in an incubator at 38 °C, for 80–100 h.

The plasma at the end of the incubation period was submitted to a gel filtration (Sephadex G-100) using the technique already described[6]. Most of the pressor activity of the plasma coincides with the elution volume of the albumin. The solutions having the highest activity were pooled and freeze-dried. The residue containing the pressor substance (VA) after dialysis against 0.9% NaCl was ready for the experiments. Usually 0.1 ml of this solution containing 10 mg of protein/ml was used. Car-



Blood pressure (B.P.) changes in a nephrectomized rat produced by i.v. injections: (1, 2, 3 and 4) of 0.3, 0.3, 0.1 and 0.2 ml, respectively, of human plasma (HP) incubated for 96 h at 38 °C; (5, 6 and 7) of 2, 1 and 0.1 mcg of bradykinin; (8) of 500 U (1 mg) carboxypeptidase B infused in 0.5 ml NaCl 0.9%; (9, 10 and 11) of 0.2, 0.15 and 0.1 ml of incubated human plasma; (12 and 13) of 0.5 and 1 mcg bradykinin.

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