ated. Only a few streaks of blood were found in the lumen. The intestinal mucosa was intact except for scattered areas of erosion.

Discussion. Lethal doses of i.v. endotoxin produced different effects in the 3 species[5,10]. In the dog massive hemorrhagic necrosis of the gastrointestinal tract, central lobular necrosis of the liver, congestion of the kidney, minimal edema of the lungs, and occasional areas of myocardial necrosis with zonal lesions occurred[9,10]. The rabbit demonstrated minimal gastrointestinal lesions, thrombosis of the hepatic vessels with necrosis, myocardial hemorrhages and necrosis, and massive hemorrhagic pulmonary edema[10]. The monkey responded with less pulmonary pathology than the rabbit and less gastrointestinal pathology than the dog and displayed no hepatic congestion[10]. In the present experiments, the pathologic changes induced in all 3 species by intrathecal endotoxin were essentially the same; massive hemorrhagic pulmonary edema, subendocardial hemorrhage and congestion of the splanchnic bed, liver, kidneys, and adrenals. These results indicate that the mechanism of death following CNS endotoxin is similar in the 3 species studied. Since intrathecal endotoxin does not induce changes which resemble those seen following i.v. endotoxin, it is unlikely that endotoxin administered i.v. acts primarily on the central nervous system.

Table I. LD_{80} *E. coli* endotoxin by i.v. and intracisternal routes

Species	Intravenous	Intracisternal
Dog	11.0 mg/kg	5.1 mg/kg
Rabbit	27.6 mgm/kg	3.5 mg/kg
Monkey	91.9 mgm/kgm	30.0 mgm/kgm

Table II. Lung/heart weight ratio (mean $\pm$ S.E.)

Species	Control	$\bar{p}$ Intracisternal endotoxin
Dog	1.23 ± 0.04	2.70 ± 0.33
Rabbit	1.74 ± 0.18	3.34 ± 0.26
Monkey	1.64 ± 0.09	2.83 ± 0.41

Zusammenfassung. Pathologische Veränderungen bei Kaninchen, Hunden und Affen nach intrazysternaler Verabreichung von Bakterienendotoxinen sind einander sehr ähnlich, während sie nach i.v. Injektion bei allen 3 Tiergruppen auffallend verschieden sind. Dies führt zur Annahme, dass die Wirkung des Endotoxins zur Hauptsache offenbar nicht über das Zentralnervensystem geht.

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The Effect of Some Anticholinesterase Agents and of Hemicholinium on the Amount of Substance P in Rabbit Brain and Gut

It has been reported that physostigmin decreased the content of substance P (SP) in gut and in brain of rabbit[1,2]. In order to elucidate the action of other anticholinesterase agents on the SP content in the abovementioned organs of the rabbit, we studied the effect of phospholine iodide and of paraoxon. Phospholine iodide, an anticholinesterase agent which does not penetrate the blood-brain barrier[3], and paraoxon, an anticholinesterase agent, which penetrates the barrier[4], were used to clarify more precisely the influence of these agents on the SP content in brain and in gut. We administered, also, hemicholinium No. 3 (HC-3) in order to inhibit cholinacetylase activity[5] and measured thereafter the concentration of SP in rabbit's small intestine and in brain.

Rabbits of both sexes were used weighing from 2–3 kg. The extracts were made from the whole of the small intestine and from the brain, cerebellum being left out. The brain and small intestine were ground and boiled in acidified distilled water. After precipitation with ammonium sulfate, SP was adsorbed on aluminium oxide and eluted with distilled water as described by PERNOW[6] and EULER[7]. Bioassay was performed on the isolated guinea-pig ileum bathed in tyrode solution which, also, contained atropine, promethazine and D-lysergic acid diethylamide. A preparation of SP (manufactured by Hoffmann-La Roche, Basle, Switzerland) containing 75 U/mg was used as a standard. The recovery was checked and the mean recoveries were of the order of 78%.

Drugs used were: phospholine iodide (dietoxyphosphoryltiocholine iodide), paraoxon (diethylparanitrophenil) and hemicholinium No. 3 (HC-3). All drugs were injected s.c. once a day. Phospholine iodide was administered for 4 days, 25 µ/kg on the first day and 12.5 µ/kg on the other 3 days. Paraoxon was administered for 2 days, 150 µ/kg on the first day and 75 µ/kg on the second day. HC-3 was injected once in dose of 2.5 mg/kg. With such a big (toxic) dose we got more uniform results than with smaller (0.5 mg/kg and 0.25 mg/kg) doses. About 1 h after 2.5 mg/kg of HC-3 the animals died with symptoms of asphyxia.

The results are shown in the Table. Phospholine iodide and paraoxon lowered the SP content in the rabbit small intestine. HC-3, on the other hand, increased the SP content in the small intestine (from 2.53 ± 0.2 U/g in control experiments to 5.70 ± 0.7 U/g). Phospholine iodide did not change the SP content in the brain, while paraoxon and HC-3 lowered it.

The present experiments are in accordance with previously reported results that physostigmine decreases the

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SP content in rabbit's small intestine and in the brain. Those anticholinesterases which penetrate the blood-brain barrier (paraoxon) decreased the concentration of SP in the brain, but those which do not pass that barrier did not change it. Both these anticholinesterases decreased the SP content in small intestine. HC-3, choline acetylase inhibitor, increased the SP content in the small intestine. It was surprising to find that this substance decreased the SP content in the brain. This effect might well be due to asphyxia produced by HC-3.

The results of the present experiments indicate that substances which affect acetylcholine metabolism can at the same time change the content of SP in the small intestine and in the brain of rabbit.

Amount of SP (U/g) in brain and small intestine of rabbit after administration of drugs

Drug	No. experiments	Brain	P	Small intestine	P
Control	5	16.0 ± 1.2		2.53 ± 0.2	
Phospholine iodide	5	16.1 ± 1.1		1.48 ± 0.15	< 0.025
Paraoxon	5	9.5 ± 1.2	< 0.05	1.55 ± 0.17	< 0.05
HC-3	5	8.0 ± 0.8	< 0.025	5.70 ± 0.7	< 0.025

Résumé. Les substances anticholinestérasiques s'avèrent capables de réduire la concentration en substance P du cerveau et de l'intestin du lapin. Cependant l'hémicholinium No 3 augmente la concentration de la substance P de l'intestin et diminue celle du cerveau.

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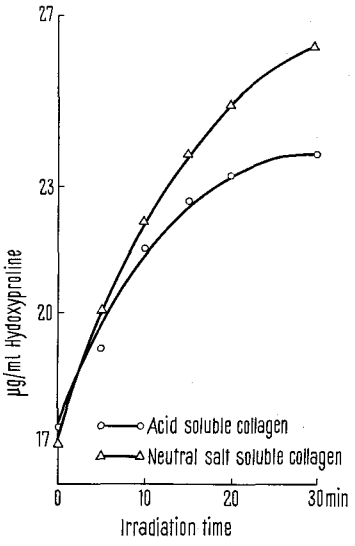
Changes in Hydroxyproline Content of Human Dermal Collagen Following UV-Irradiation in vitro

In contrast to senile skin, actinic elastosis reveals a decrease in hydroxyproline content of connective tissue[1]. This observation needs further explanation; there might be a true decrease in hydroxyproline content of collagen and elastin damaged by chronic actinic influence, or this decrease might only be a relative one, caused by the participation of other substances which do not contain hydroxyprolin in the formation of the pathologic ('elastotic') material. The latter assumption seems more likely. As a contribution to this still unsolved problem in actinic elastosis, changes in hydroxyproline content of human dermal collagen were investigated following UV-irradiation in vitro.

Material and methods. Dermal tissue of human abdominal skin was separated from adherent subcutis and epidermis, minced, freeze-dried and extracted with either 1% acetic acid ('acid soluble collagen') or 0.05 *M* phosphate buffer pH 7.2 ('neutral salt soluble collagen')[2]. Irradiation of the 2 collagen solutions with UV-light (Hanau S 200, distance 20 cm) was performed in a petri dish under steady stirring and cooling. In 5 min intervals for $^1/_2$ h, samples were withdrawn. Hydroxyproline was determined according to the micromethod of Stegemann[3]. The values given were the mean of triple investigations in 5 irradiation experiments.

Results. Irradiation with UV-light causes an increase in hydroxyproline content of acid soluble and neutral salt soluble collagen. Control experiments with UV absorptive filters only gave insignificant changes. The relative increase of hydroxyproline content was higher in acid soluble collagen (+ 50%) than in neutral salt soluble collagen (+ 40%). For details see Figure.

Comment. Hydroxylation of protein- or peptide-bound proline can be effected either by oxygen directly or via the formation of hydrogen peroxide[4,5]. Both mechanisms might be involved in the experiments presented here. It must be assumed from the data collected in other studies[6] that the energy reaching the dermal connective tissue in vivo is sufficient to cause hydroxylation of bound proline. As UV-irradiation in vitro increases hydroxyproline content of collagen, it seems most unlikely that UV-irradiation in vivo produces an opposite effect.



Increase in hydroxyproline content of acid soluble and neutral salt soluble collagen.

Zusammenfassung. UV-Bestrahlungen von menschlichem dermalem Kollagen führen in vitro zu einer Zunahme des Hydroxyprolingehaltes.

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