demonstrable to fragments of rabbit Achilles tendon. b) *Factors in whole blood* which are missing from platelet-rich plasma, as shown in specimens perfused with citrated preparations. Whether this is HELLEM's[8] factor 'R' of red cells which was later identified as ADP[9], or a labile protein inactivated during preparation of platelet-rich plasma, or a rheologic phenomenon remains to be established.

These considerations are in contrast to the platelet-collagen adhesion reaction which occurs equally well in whole blood or platelet-rich plasma with or without divalent cation. Studies on the mechanism of platelet adhesion to collagen is thus not sufficient for complete understanding of platelet hemostatic reactions; other biologic materials, such as non-collagenous microfibrils, and factors influencing platelet adhesion, such as red cells, deserve additional attention.

Zusammenfassung. Das subendotheliale Gewebe von Kaninchenaorten besteht zur Hauptsache aus Mikrofibrillen, Elastin und amorphem Material. Die Adhesion von Blutplättchen an subendothelialem Gewebe ist von der Präsenz von Erythrozyten und chelierbaren Ionen abhängig. Dies steht im Gegensatz zur Adhesion von Plättchen an Kollagen.

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Effect of Stimulation on Synaptic Vesicles in the Superior Cervical Ganglion of the Cat

Physiological[1,2], morphological[3,4] and biochemical[5] evidence suggests that synaptic vesicles may play an important role in the storage and release of neurotransmitter substances. Furthermore, several investigators[6–8] have recently reported that prolonged (15–90 min) stimulation can under certain conditions cause a reduction in the number of vesicles in cholinergic nerve terminals. In some of these latter experiments evidence of transmission failure was obtained and this phenomenon appeared to be related to the depletion of synaptic vesicles. However, no attempt was made to correlate these changes in the vesicle population with the tissue content of acetylcholine (ACh).

Since we[9] had previously demonstrated that preganglionic stimulation at 60/sec for 4 min could reduce the ACh content by about 30%, we thought it would be of interest to ascertain what effects such a short period of stimulation would have on synaptic vesicles in the cat's superior cervical ganglion. If these vesicles are the storage sites for ACh, then one might expect that a significant depletion of ACh would be accompanied by a corresponding reduction in the number of vesicles.

Methods. Cats weighing 1.5–2.5 kg were anesthetized with α-chloralose i.p. (80 mg/kg). Superior cervical ganglia were exposed by careful dissection so that the natural blood supply to these tissues was preserved. Preganglionic nerve trunks were stimulated at a frequency of 60/sec with pulse durations of 2 msec. The voltage (5–10 V) was adjusted to obtain a maximum response as judged by the degree of mydriasis and the isometric contractile response of the nictitating membrane. Ganglia were fixed by perfusion via the carotid artery with 2% (w/v) glutaraldehyde and were post fixed in 1% (w/v) osmium tetroxide.

Results and discussion. Electronmicrographs of control unstimulated nerve endings are presented in Figures A and B. These pictures reveal presynaptic nerve terminals with their normal content of vesicles and Figure B also illustrates the typical structure of mitochondria which are frequently found in these nerve endings[10]. Preganglionic stimulation at 60/sec for 4 min did not cause any apparent failure of ganglionic transmission, but did induce marked alterations in the ultrastructure of nerve endings and a 30% reduction in the ACh content. Invariably this brief period of stimulation caused a depletion of synaptic vesicles and many of those remaining appear to have lost their characteristic conformation (Figures C and D). In addition, these stimulation parameters frequently caused the swelling and disruption of mitochondria in presynaptic nerve endings (compare mitochondria in Figures B and D). Other investigators[7,8] have also reported similar changes in mitochondria, but they stimulated nerves for a longer period of time (15–90 min). In this regard, we found that stimulation for 4 min at 5/sec did not reduce the ACh content nor did this lower frequency of stimulation induce significant alterations in the fine structure of nerve terminals.

In several preliminary experiments ganglia, which had previously been stimulated at 60/sec for 4 min, were allowed to rest for 2 min in order to determine whether the number of vesicles would increase toward control values. Although the ACh content was restored during this rest period, many nerve endings still had not recovered their quota of vesicles. In view of these latter results the data must be interpreted with caution.

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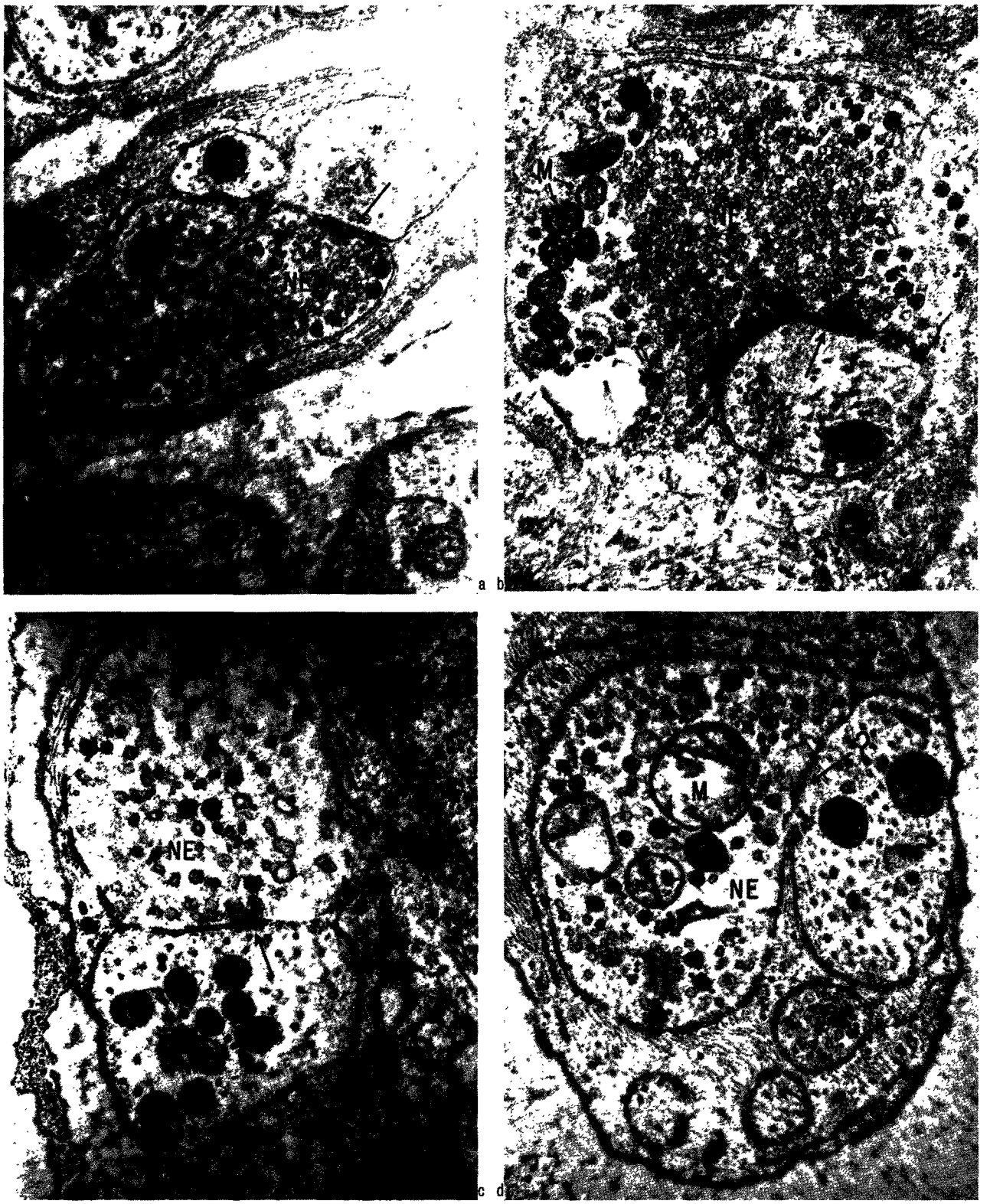
In conclusion, the function of synaptic vesicles in the chemical transmission of nervous impulses is still in doubt. Further experiments are required to clearly establish the reversibility of the observed changes of these vesicles; the alterations in the number, shape and location of vesicles should also be shown to be related to the functional status and ACh content of cholinergic nerves. Merely demonstrating that synaptic vesicles can



Electronmicrographs of preganglionic nerve terminals of the superior cervical ganglion of the cat. Nerve endings from unstimulated ganglia are depicted in A and B. The effects of 4 min of preganglionic stimulation at 60/sec on the ultrastructure of nerve endings are shown in C and D. Arrows indicate synaptic regions; M, mitochondria; NE, nerve ending. ×25,000 in each case.

be depleted by procedures which cause transmission failure or a reduction in ACh stores does not necessarily imply that these vesicles are intimately involved in the storage and release of ACh[11].

Résumé. La stimulation préganglionique à 60/sec pendant 4 min produit un épuisement des vésicules synaptiques et une réduction de 30% du contenu de l'acétylcholine. Après cette période de stimulation, on observe fréquemment l'enflure de l'éclatement des mitochondries aux extrémités des nerfs. Ces changements dans l'ultrastructure de ces dernières ne se sont pas encore montrés reversibles lorsque l'on laisse par la suite les ganglions se reposer pendant plusieurs minutes.

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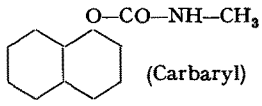
Pharmacological Effects of Carbaryl II. Modification of Serotonin Metabolism in the Rat Brain

In a previous paper[1] it was reported that the administration of a mixture of DDT, parathion and carbaryl (L-naphthyl-N-methylcarbamate) induced an increased elimination of urinary 3-methoxy-4-hydroxy-mandelic acid (VMA) and 5-hydroxy-3-indolylacetic acid (5-HIAA) in the rabbit. It was postulated that a stress mechanism is involved, featuring an increased synthesis rate of the catecholamines and Serotonin respectively. HASSAN[2] demonstrated an increased sympathoadrenergic activity in the rat, following the oral administration of carbaryl. This increased activity involved increased synthesis of norepinephrine periferally (and probably centrally) with concomitant increase of urinary VMA excretion.



(Carbaryl)

The purpose of the present investigation was to study the effect of a single carbaryl dose on serotonin metabolism in the rat brain.

Male albino rats (Holtzman) weighing 150–180 g were used in this study. All animals were housed in groups for several weeks, and fed a standard diet of Purina laboratory chow. Carbaryl was administered orally as a suspension in peanut oil at a single dosage of 60 mg/kg. Control rats received only peanut oil. Animals were killed by decapitation, and the brain was quickly removed and extracted. All determinations were made on whole brain. Serotonin was determined by the procedure of BOGDANSKI[3] and 5-HIAA by the method of GIACALONE and VALZELLI[4]. The concentration of corticosterone in the plasma was estimated by a fluorescence method[5]. The Aminco Bowman Spectrophotofluorometer was used for all fluorescent measurements.

The results of the study on the level of 5-HT and 5-HIAA in the brain are shown in the Table. The concentration of the amine and its metabolite increased significantly after 2, 4 and 6 h following carbaryl administration. After 24 h the level of both substances returned to normal.

In order to investigate the effect of carbaryl on brain 5-HT formation, endogenous stores of the amine were previously depleted by inhibition of its synthesis. Carbaryl was given 72 h after *p*-chlorophenylalanine (PCPA) administration and animals were sacrificed 4 h later. The amine level was significantly higher when compared with PCPA control value (Figure 1). Reserpine-pretreated animals showed the same trend. Plasma corticosterone level was also increased by about 125%, one hour after the oral administration of the carbamate (Figure 2).

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Effect of a single carbaryl dose on brain 5-HT and 5-HIAA

Hours after Carbaryl	5-HT		5-HIAA		Ratio 5-HIAA 5-HT
	Concentration μg/g ± S.E.	% Increase	Concentration μg/g ± S.E.	% Increase	
0	0.61 ± 0.05	–	0.25 ± 0.02	–	0.41
2	0.78 ± 0.07	28	0.30 ± 0.02	20	0.38
4	0.78 ± 0.08	28	0.31 ± 0.03	24	0.40
6	0.80 ± 0.07	31	0.31 ± 0.03	24	0.39
24	0.60 ± 0.06	–	0.24 ± 0.02	–	0.40

Carbaryl dose 60 mg/kg. Results are mean of groups of 5–8 animals ± S.E.