in 2 important respects: (1) Tetrodotoxin (10^{-5}), which is ineffective in blocking the normal spikes of taenia coli, does block the Ca-free spikes completely (Figure 2). (2) Manganese ions, which block the normal activity in concentrations of 0.5–1 mM [4–6], inhibit the Ca-free spikes in a concentration of only 0.01 mM (Figure 3).

On the basis of the ionic theory, the only explanation of the Ca-free spikes seems to be that sodium ions are the charge carriers producing the depolarizing current of the spike. The positive tetrodotoxin effect on these spikes is consistent with this view [9,10]. The fact that the Ca-free spikes are about 100 times more sensitive to manganese than are the normal spikes of taenia coli indicates that manganese does not inhibit the spikes by competition with calcium in its function as the charge carrier of the spike. It cannot be decided whether the manganese inhibition under 'Ca-free' conditions, when small amounts of Ca are still present in the tissue, is independent of Ca, or perhaps a competition with Ca in another function. The inhibitory effect of manganese ions on spikes can no longer be interpreted as an indication that calcium is the charge carrier of these spikes.

With these results, some questions about the spike mechanism in intestinal smooth muscle are again moot. The fact that sodium spikes can be elicited relatively easily seems to indicate that sodium fluxes may also be involved in the spike generation under normal conditions. The most interesting aspect of our results seems to be that the differences in spike mechanism of various smooth muscles (see KEATINGE [7]) need not be interpreted as fundamental differences, since in one tissue 'calcium spikes' can be converted to 'sodium spikes' by simple procedures.

Zusammenfassung. An der glatten Darmmuskulatur (Taenia coli) des Meerschweinchens konnten nach völligem Entzug von Kalzium (und Magnesium) aus der Nährlösung über lange Zeit Spikes gemessen werden. Diese 'kalziumfreien Spikes' werden, im Gegensatz zu den Spikes unter normalen Bedingungen, durch Tetrodotoxin 10^{-5} blockiert, und sie werden wie die normalen Spikes mit 100fach niedrigerer Schwelle durch Manganionen blockiert. Es wird angenommen, daß Na-Ionen die für diese Spikes verantwortlichen Ladungsträger sind.

K. GOLENHOFEN and P. PETRÁNYI

Physiologisches Institut der Universität, 355 Marburg/Lahn (Germany), 14 October 1968.

[9] H. S. MOSHER, F. A. FUHRMAN, H. D. BUCHWALD and H. G. FISCHER, Science *144*, 1100 (1964).
[10] C. Y. KAO, Pharmac. Rev. *18*, 997 (1966).

Degeneration of the Peripheral and Central Nervous System in Vitamin-B_{12}-Deficient Monkeys

Following on the report of OXNARD and SMITH [1] on reduced serum-vitamin-B_{12} levels and neurological degeneration in captive monkeys, further qualitative and quantitative investigations have been carried out, with particular reference to the peripheral nerve lesions and to the effect of treatment.

Materials and methods. 43 monkeys in the colony of the Anatomy Department, University of Birmingham, were studied. There were 40 rhesus monkeys (*Macaca mulatta*), 2 patas monkeys (*Erythrocebus patas*), and 1 baboon (*Papio anubis*); 2 were males and 41 were females of which 10 were pregnant. The animals were grouped according to duration of captivity and diet: group I comprised 12 monkeys kept in captivity from 11 months to 10 years on a standard vegetarian diet; group II comprised 14 monkeys captive from 11 months to almost 19 years, originally fed vegetarian diets but subsequently given a series of injections of vitamin B_{12} followed by a normal diet for periods that varied from 6 months to 4 years; and control group III comprised 17 recently captive monkeys given vitamin B_{12} since arrival in the colony, 14 animals having been in captivity for less than 1 month, and the other 3 animals for 6, 7 and 15 months respectively. The amounts of vitamin B_{12} in the serum were estimated in 38 cases by the bioassay technique with *Euglena gracilis*; OXNARD [2] found the mean total level to be 271 µg/ml in recently captive monkeys. In group I 10 animals had deficient levels of the vitamin in the serum (mean total level 79 µg/ml); in group II 12 animals had high serum levels (mean total > 900 µg/ml); and in group III the serum levels were normal, except for 2 pregnant monkeys that had low readings.

Results. The results are given in the Table.

A high incidence of histological lesions was found in animals of groups I and II and 5 animals showed overt paralysis (see Table). It should be noted that this does not represent the true incidence of paralysis in deficient animals, which is much lower: paralysed animals were selected because of the paralysis and in order to try and assess the effect of treatment. In the spinal cord the changes resembled those of human subacute combined degeneration, and were more severe in the paralysed monkeys; cerebral lesions were found in 5 animals. Frozen sections of the sciatic and popliteal nerves showed sudanophilic degeneration in 21 animals (the median and ulnar nerves were normal in all 43 cases). Segmental demyelination, usually with remyelination, was seen in teased peripheral nerve fibres in every animal that showed sudanophilic degeneration (see Figure); in a few animals there was also axonal (wallerian) degeneration.

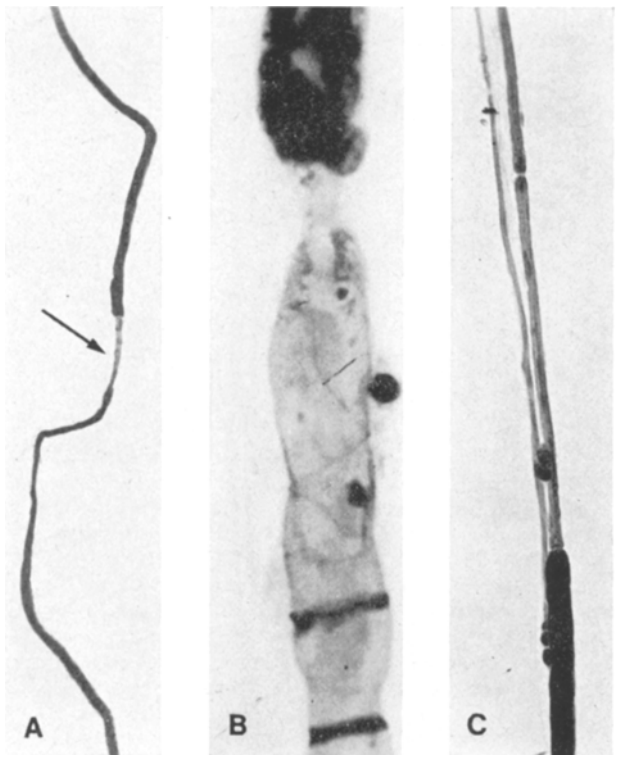
In group III minimal lesions were found in 4 animals. Two of these were pregnant and had low serum-vitamin-B_{12} levels (pregnancy depresses the serum vitamin B_{12} in monkeys: OXNARD [2]) and a third animal had a low-normal level; though the previous dietary history of these 3 animals was unknown, their nervous systems could have been affected before they arrived in captivity. The fourth animal had been captive for 15 months (more than twice as long as any other animal in group III) and

[1] C. E. OXNARD and W. T. SMITH, Nature *210*, 507 (1966).
[2] C. E. OXNARD, Nature *201*, 1888 (1964).

Incidence of neurological lesions in groups I–III

Group (total No. of animals)	Functional status		Histological findings						
	Normal	Paralysed	No. of animals with lesions	Distribution of lesions					
				Cerebrum	Cerebellum	Brain stem	Spinal cord	Posterior nerve roots and/or posterior root ganglia	Peripheral nerves (lower limbs)
I (12)	11	1	12	3	1	3	12	3	10
II (14)	10	4	13	2	1	1	11	6	11
III (17)	17	0	4 (slight)	0	0	0	2	1	3

The nerve roots and the posterior root ganglia were not examined in 2 cases in group I, neither was the brain-stem in one of these cases.



(a) A dissected nerve fibre showing an incompletely remyelinated short segment (arrow). Osmium stained. × 100. (b) A dissected nerve fibre showing a widened node of Ranvier and part of a demyelinated internode in which the Schmidt-Lantermann incisures are clearly seen. Osmium stained. × 720. (c) Two dissected nerve fibres. One of these shows part of a normal internode and 2 thin remyelinated segments of reduced internodal length. Phagocytic cells adhere to the surface of the fibres. Osmium stained. × 250.

by an oversight its serum level was not examined; previous studies[1] showed that reduced levels occur rapidly and neural lesions could result in this period if, as can happen, the animal had not been receiving its proper diet.

Nerve fibre counts. Group I (vitamin B_{12}-deficient-animals), group II (long stay non-paralysed treated animals), and group III (recently captive animals), showed similar average mean fibre density counts/mm² of intraperineurial area in myelin-stained transverse sections of sciatic and popliteal nerves; this was taken as evidence that axonal degeneration was not the main lesion. The only average values that clearly differed either individually or on the whole from the average values of the other groups, were those of the 4 paralysed treated animals in group II, which were reduced; Student's *t*-test showed that subgroups A (10 non-paralysed animals) and B (4 paralysed animals) of group II, were significantly different ($P = 0.05$).

Discussion. Histological evidence of reparative changes clearly attributable to vitamin-B_{12} therapy, has not been found in either the central or the peripheral nervous system. Though we have previously found remyelination in treated animals[3], we have now been able to show that remyelination and occasional axonal regeneration can occur in deficient as well as treated monkeys. Some functional improvement was noted in the latter, which may result from arrest of the degenerative process and recovery of suppressed nerve function.

The findings of Oxnard and Smith[1] in a small number of cases that suggested a greater involvement of the distal parts of the nerves of the lower limbs, possibly due to a 'dying back' of the peripheral parts of the axons, has not been confirmed. The distribution pattern of the lesions found in the present study, is consistent with random involvement of Schwann cells rather than selective damage to the distal axons. It was not possible to ascertain whether the occasional nerve fibre that showed axonal (wallerian) degeneration had been affected primarily or had degenerated as a consequence of Schwann-cell disease.

None of our monkeys showed lesions in the gastrointestinal tract such as mucosal atrophy or parasitic infestation to account for the deficiency of vitamin B_{12}. The possibility that other nutritional defects were also present cannot be entirely disregarded though the similarity to human subacute combined degeneration and the functional improvement noted after treatment, strongly suggest that lack of vitamin B_{12} in the animals' diets was the main causal factor[4].

[3] C. E. Oxnard, W. T. Smith and I. Torres N, Acta neurol. latinoam., in press.

[4] We are indebted to the Agricultural Research Council (Grant No. 40319) and to the Ministerio de Sanidad y Asistencia Social through the Universidad de Oriente, Venezuela for generous financial support.

Resumen. Monos cautivos mantenidos en una dieta vegetariana presentaron niveles séricos de vitamina B_{12} reducidos y lesiones en el sistema nervioso central semejantes a las de la «degeneración combinada sub aguda» del humano. Los nervios periféricos mostraron desmielinización segmentaria, compatible con un compromiso de las células de Schwann al azar. Usualmente hubo remielinización segmentaria, asociada a veces con degeneración axónica (walleriana). No se encontró evidencia de remielinización o de regeneración axónica que pudiera ser atribuída totalmente al tratamiento con vitamina B_{12}.

I. TORRES N., W. TH. SMITH and CH. OXNARD

Departments of Anatomy and Pathology, University of Birmingham (England), 20 October 1968.

Effect of Prostaglandin E_1 on the Strychnine-Induced Convulsion in the Mouse

Prostaglandins were shown to be released from central nervous system and to have some effects on the brain and spinal cord functions[1–5]. In unanesthetized cats, intraventricular injection of prostaglandin E_1 (PGE_1) (7–20 μg/kg) decreased spontaneous activity. However, it reduced spontaneous activity only slightly when given i.v.[1]

In a recent study it was shown that PGE_1 usually caused a delayed long-lasting inhibition of monosynaptic reflex ventral root potentials[6]. PGE_1 was also demonstrated to have a decamethonium-like neuromuscular blocking action in anesthetized cats[7].

In the present investigation the effect of PGE_1 on the convulsion induced by strychnine was studied in mice.

Material and methods. The experiments were carried out on adult, white, male mice from a homogenous strain weighing 20–31 g. The animals were divided into 3 groups of 12 mice each. The first group was injected 2 mg/kg strychnine sulfate i.p. in 0.2 ml saline. This group served as control. The second group was injected 20 μg/kg PGE_1 and the third group 30 μg/kg PGE_1 i.p. (in 0.2 ml saline), 1 min before strychnine administration. The animals were exposed to the same stimuli during the experiments.

The interval between strychnine injection and onset of the first convulsion was recorded. The duration of convulsions and the time of the last convulsion were also recorded. The results were evaluated statistically using Student's *t*-test.

Effect of PGE_1 (20 or 30 μg/kg i.p.) on the convulsions produced by 2 mg/kg i.p. strychnine

	Interval between injection of strychnine and convulsion (min)	Duration of convulsion (min)	Time of last convulsion following strychnine injection (min)
Control (n = 12)	3.25 ± 0.036[a] (3.12–3.45)[b]	1.89 ± 0.077 (1.45–2.16)	5.32 ± 0.042 (5.10–5.50)
After treatment with 20 μg/kg i.p. PGE_1 (n = 12)	5.24 ± 0.034 (5.10–5.50) $p < 0.005$	4.96 ± 0.1 (4.32–5.35) $p < 0.005$	10.34 ± 0.04 (10.11–10.51) $p < 0.005$
After treatment with 30 μg/kg i.p. PGE_1 (n = 12)	7.62 ± 0.07 (7.40–8.05) $p < 0.005$	9.60 ± 0.065 (9.33–10.15) $p < 0.005$	17.51 ± 0.09 (17.16–18.20) $p < 0.005$

[a] Mean ± S.E. of the mean, [b] range, *p*, statistical significance of difference between treated and corresponding controls.

Results. The appearance of the convulsions is significantly delayed in mice treated with 20 μg/kg PGE_1 i.p. In this group, an increase was found in the duration of convulsion and in the time elapse till the last convulsion. The controls and the 20 μg/kg i.p. PGE_1 treated mice did not survive the convulsion. In the 30 μg/kg i.p. PGE_1 treated group, however, all mice survived the convulsion, though the duration of convulsion was found to be longer than in the first and second groups (Table).

Discussion. The antagonistic action of PGE_1 to the lethal effect of strychnine as observed in the third group suggests a dose dependent interaction between the 2 drugs. Several explanations are possible to account for this interaction, including an interference with the absorption and distribution of strychnine. In view of the fact that strychnine increases the level of excitability in spinal neurons by selectively blocking supraspinal inhibition[8], PGE_1 might counteract this disinhibition by strychnine. A similar suggestion was made by DUDA et al.[6] regarding the inhibitory action of PGE_1 on monosynaptic reflexes in cats. This antagonistic effect can also be explained by a neuromuscular action of PGE_1, as observed by KHAIRALLAH et al.[7,9].

Zusammenfassung. Es wurde gezeigt, dass PGE_1 gegen durch Strychnin hervorgerufene Krämpfe deutlich antagonistisch wirkt.

S. DURU[10] and R. K. TÜRKER

Department of Pharmacology, Faculty of Medicine, University of Ankara (Turkey), 6 November 1968.

[1] E. W. HORTON, Br. J. Pharmac. Chemother. *22*, 189 (1964).
[2] E. W. HORTON and I. H. M. MAIN, Int. J. Neuropharmac. *4*, 65 (1965).
[3] G. L. AVANZIO, P. B. BRADLEY and J. J. WOLSTENCROFT, Br. J. Pharmac. Chemother. *27*, 157 (1966).
[4] E. W. HORTON and I. H. M. MAIN, Br. J. Pharmac. Chemother. *30*, 568 (1967a).
[5] E. W. HORTON and I. H. M. MAIN, Br. J. Pharmac. Chemother. *30*, 582 (1967b).
[6] P. DUDA, E. W. HORTON and A. MCPHERSON, J. Physiol., Lond. *196*, 151 (1968).
[7] P. A. KHAIRALLAH, I. H. PAGE and R. K. TÜRKER, Archs int. Pharmacodyn. Thér. *169*, 328 (1967).
[8] D. W. ESPLIN and B. ZABLOCKA, in *The Pharmacological Basis of Therapeutics*, 3rd edn (Ed. L. S. GOODMAN and A. GILMAN; Macmillan Co., New York 1965), p. 345.
[9] Acknowledgments. We are indebted to Prof. Dr. D. A. VAN DORP, Unilever Research Laboratorium, Vlaardingen (Netherlands) and to Upjohn Company, Kalamazoo, Michigan, USA for supplying crystalline pure prostaglandin E_1.
[10] Fellow in the Department of Pharmacology, School of Medicine, University of Hacettepe, Ankara, Turkey.