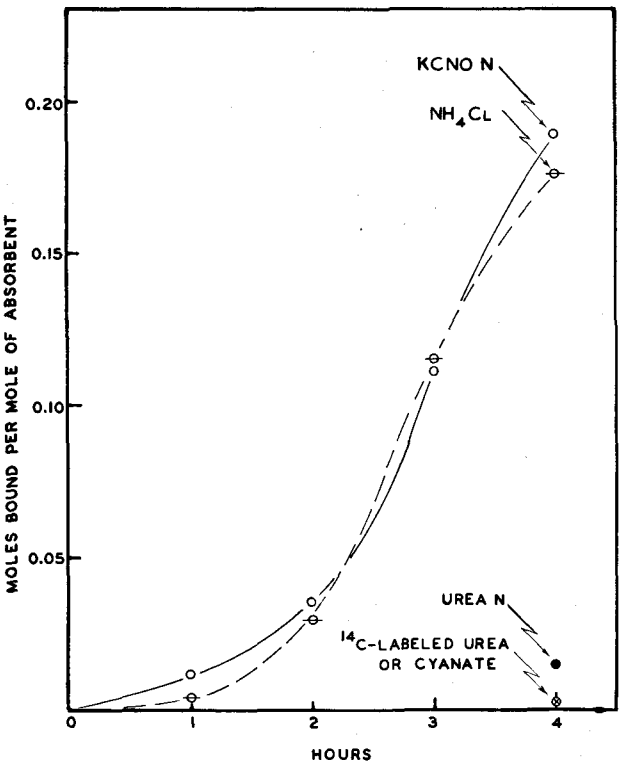
As shown in the Figure, ammonium or cyanate ion binding as determined by N analyses during equilibrium dialysis occurred more rapidly and in greater amount than did binding of urea. When determined by isotope, however, no binding of the carbon atoms of urea or KOCN was detectable. It was evident, therefore, that neither urea N nor cyanate were bound to oxidized starch as unchanged entities.

Discussion. The data presented here demonstrated that binding of urea to oxidized starch was negligible, but that binding of nitrogenous products of urea hydrolysis proceeded rapidly. Since the carbon atom of cyanate ion was not bound, and the degrees of molar binding of anionic cyanate and cationic ammonium ion were similar it was apparent that complete decomposition through cyanate was essential for N binding by oxidized starch. The kinetics of urea conversion in water to cyanate, and conversion of cyanate to CO_2 and ammonium ion have been reviewed by HAGEL et al.[5]. In the presence of polyaldehyde it seemed likely that the equilibrium was shifted towards ammonium ion formation:

$$(NH_2)\,CO \rightleftharpoons NCO^- + NH_4^+$$
$$NCO^- + H_2O \rightleftharpoons HCO^-_3 + NH_4^+$$

→ N-aldehyde oxidized starch complex

The above observations thus accounted for the long time required to demonstrate 'urea' binding by aldehyde starch preparations. Furthermore, in view of these data, a proposed mechanism for removal of fecal N by such absorbents must include conversion of gut urea into ammonium ion, a phenomenon, well characterized in man[3,6]. While the complete mechanism for increased fecal N removal by carbohydrates may not necessarily involve N-binding[7], the fact that uremic patients are unable to hydrolyze more urea than normal individuals[8] may yet leave an important therapeutic role for an innocuous gastrointestinal ammonium ion binding agent.



Time course of solute binding by oxidized starch during equilibrium dialysis. Each prospective ligand was 0.04 *M* and dialyses were performed with stirring at room temperature as described in Methods.

Summary. By use of ^{14}C label it was demonstrated that apparent binding of urea N to polyaldehyde starch was probably preceeded by hydrolysis to ammonium ion. Thus direct urea binding was not the mechanism through which ingested polyaldehyde starch might increase fecal N excretion in uremic patients.

I. J. STERN, R. S. IZZO, Z. Y. JO WANG and W. E. BESCHORNER

Drug Metabolism Section, Pharmaceutical Research and Development Department, Travenol Laboratories, Morton Grove (Illinois 60053, USA), 29 April 1975.

[5] P. HAGEL, J. J. T. GERDING, W. FIEGGEN and H. BLOEMENDAL, Biochim. biophys. Acta *243*, 366 (1971).
[6] M. WALSER and L. J. BODENLOS, J. clin. Invest. *38*, 1617 (1959).
[7] N. K. MAN, T. DRUEKE, J. PARIS, C. ELIZALDE, M. RONDON, J. ZINGRAFF and P. JUNGERS, Proc. Eur. Dialysis Transplant. Ass. *10*, 143 (1973).
[8] M. WALSER, J. clin. Invest. *53*, 1385 (1974).

Further Investigations into the Effects of Baclofen (Lioresal) on the Isolated Spinal Cord

In a number of neural preparations, the effects of baclofen (Lioresal), an anti-spastic drug, are different from those of γ-aminobutyric acid (GABA), a putative inhibitory transmitter, although the two substances share a similar chemical structure[1–4]. In a previous report, it was shown that baclofen and GABA reduce the spontaneous acetylcholine (ACh) release from the isolated spinal cord but that the extent and time course of their action are different[4]. The effect of these two compounds has now been tested both on the spinal root potentials and on the electrically-evoked release of ACh, which is thought to be the neurotransmitter of motor axon collaterals[5].

Methods. Frogs (*R. temporaria*) were used. The spinal cord was removed, hemisected sagitally and placed in a 500 µl bath at 14 °C. The ventral root potential (VRP) evoked by orthodromic stimulation of the corresponding dorsal root, or the dorsal root potential (DRP) evoked by antidromic stimulation of the corresponding ventral root were recorded with Ag/AgCl electrodes and displayed on a storage oscilloscope and on a pen recorder as already described[6,7]. The ACh release was measured every 10 min as previously reported[4,6].

[1] D. R. CURTIS, C. J. A. GAME, G. A. R. JOHNSTON and R. M. MCCULLOCH, Brain Res. *70*, 493 (1974).
[2] J. DAVIES and J. C. WATKINS, Brain Res. *70*, 501 (1974).
[3] R. A. DAVIDOFF and E. S. SEARS, Neurology, Minneapolis *24*, 957 (1974).
[4] A. NISTRI and A. CONSTANTI, Experientia *31*, 64 (1975).
[5] J. F. MITCHELL and J. W. PHILLIS, Br. J. Pharmac. Chemother. *19*, 534 (1962).
[6] A. NISTRI, J. Physiol., Lond. *246*, 32P (1975).
[7] A. NISTRI and A. CONSTANTI, Eur. J. Pharmac. *31*, 377 (1975).

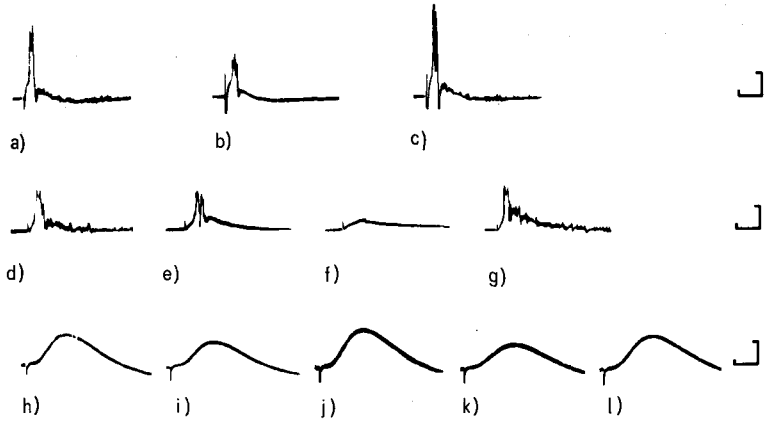


Fig. 1. Effect of GABA (10^{-4} *M*) and baclofen (2×10^{-4} *M*) on the VRP: a) and d) controls; b) GABA (5min); c) 10 min after wash; e) and f) baclofen (3 and 4 min respectively); g) 60 min after wash. Effect of GABA and baclofen (doses as above) on the DRP: h) control; i) GABA (5min); j) 10 min after wash; k) baclofen (5 min); l) 70 min after wash. Calibrations: a)–c) 1 mV, 20 msec; d)–g) 2 mV, 20 msec; h)–l) 0.5 mV, 20 msec.

Results. The effect of GABA (10^{-4} *M*) and baclofen (2×10^{-4} *M*) on the VRP and DRP is shown in Figure 1. Both compounds depressed these potentials. However, while baclofen hyperpolarized the ventral and the dorsal root, GABA depolarized the dorsal and the ventral root, although occasional hyperpolarizations of the ventral root were also encountered. The preparations recovered within 5 min from the application of GABA and after 60–70 min from baclofen.

The ACh release from the frog cord was studied during stimulation of the ventral or dorsal root and the results are shown in Figure 2. Dorsal root stimulation (1 Hz for 10 min) was not accompanied by a change in the ACh output as already reported[5,6]; baclofen (2×10^{-4} *M*), when applied to the bath during the 10 min period of dorsal root stimulation, reduced ACh output. Conversely, GABA (10^{-4} *M*) did not vary ACh release following this type of stimulation.

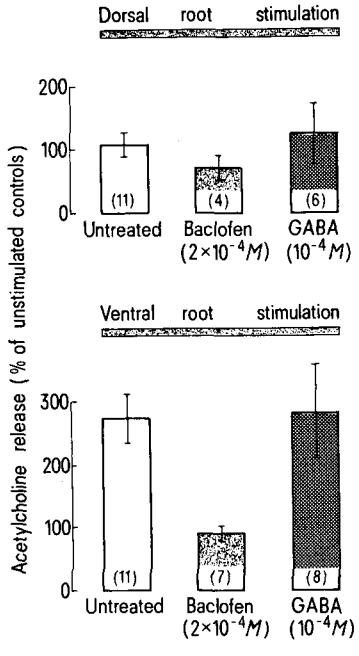


Fig. 2. Effect of GABA and baclofen on the electrically evoked ACh release from the frog spinal cord. Above: ACh release following 10 min of dorsal root stimulation (1 Hz; 0.1 msec; supramaximal voltage) in control (open column), baclofen-treated (dotted column) or GABA-treated (hatched column) preparations. Below: ACh release following 10 min of ventral root stimulation (parameters and symbols as above). GABA or baclofen were added to the bathing fluid when the electrical stimulation started. In untreated cords no decay in VRPs or DRPs over a 10 min period was seen. The results reported are mean ± SEM of the number of experiments in brackets and are expressed as the % of the preceeding unstimulated control values. The average ACh release from unstimulated spinal cords was 16.9 ± 2.0 ng/ml/10 min.

Since it has been suggested the baclofen might act by stimulating catecholamine receptors[1,2], the effect of noradrenaline (NA) and adrenaline (A) (the latter being the physiological catecholamine of the frog nervous tissue[8]) on the unstimulated output of ACh was next studied. The ACh release (mean ± SEM) was 55.1 ± 10.5, 78.2 ± 11.3 and 39.4 ± 10.3% of the control values 10, 20 and 30 min respectively after the addition of A (1.5×10^{-6} *M*) to the Ringer bathing the cord. It then returned to the control level. NA (5×10^{-6} *M*) was much less effective in depressing ACh release than A and a 33.1 ± 10.2% decrease was seen only 30 min after the application of NA, with recovery in the following sample.

Discussion. Although GABA and baclofen depressed both the VRP and the DRP, their effect was brought about by different mechanisms, since the first usually depolarized the root and the second hyperpolarized it. On the basis of experiments carried out in Mg^{2+}-treated frog spinal cords to suppress interneuronal activity, it was concluded that the action of baclofen on the VRP and DRP was mainly indirect through interneuronal circuits[3]. However, baclofen blocked the release of ACh following antidromic ventral root stimulation; this finding shows that the drug may have direct presynaptic effects on the terminals of the motor axon collaterals by reducing the release of their transmitter. GABA did not change the release produced by ventral root stimulation; therefore an action of this amino acid on the motorneurone terminals would not appear to be present.

The spontaneous ACh output, which would mostly result from the tonic activity of motorneurones, has already been shown to be reduced by GABA and baclofen[4] as a consequence of an action either on interneurones

[8] B. B. Brodie and D. F. Bodanski, in *Progress in Brain Research* (Eds. W. A. Himwich and H. E. Himwich; Elsevier, Amsterdam 1964), p. 234.

or on motorneurones themselves. The reduction produced by baclofen was smaller and had a time course different from that produced by a low dose of A, the physiological catecholamine of the amphibian nervous system[8]. This suggests that baclofen does not act by stimulating catecholamine receptors. In conclusion, the depression of motorneurone activity induced by baclofen appears to be the result of an action on the motorneurone nerve endings and on interneuronal mechanisms[3]. These effects may be relevant to the mode of action of baclofen as an antispastic agent[9].

[9] I thank Prof. J. P. QUILLIAM for his encouragement and Ciba-Geigy laboratories for the gift of baclofen. The technical assistance of Miss ALISON ROBSON who was supported by the Wellcome Foundation is gratefully acknowledged.

[10] Present address: Institute of Pharmacology, University of Florence, Viale Morgagni 65, I-50134 Firenze (Italy).

Riassunto. Il GABA e il baclofen (Lioresal), un farmaco usato nella terapia della spasticitá, riducono i potenziali registrati dalle radici dorsali e ventrali del midollo spinale di rana in vitro. Il baclofen riduce anche la liberazione di acetilcolina spinale prodotta dalla stimolazione antidromica delle radici ventrali. Questo effetto suggerisce un'interazione del baclofen con le terminazione nervose dei motoneuroni da cui l'acetilcolina viene liberata. Una depressione dell'attivitá dei motoneuroni spinali puó in parte spiegare il meccanismo di azione di tale farmaco.

A. NISTRI[10]

Pharmacology Department, St. Bartholomew's Hospital, Medical College, University of London, Charterhouse Square, London E.C.1M 6BQ (England), 29 April 1975.

Penetration of C^{14}-Labelled Rifampicin into Primate Peripheral Nerve

Since the first reports on the use of Rifampicin in clinical leprosy, numerous authors have attested to its rapid clinical and bactericidal action[1–7]. However, in view of the known tendency of the leprosy bacillus to multiply and persist in human nerve, and in view of the difficulty of assessing the action of Rifampicin in the nerves of patients (serial biopsies being of necessity limited) it was thought important to study the penetration of this drug into the peripheral nerves of a primate. After dissolving in propylene glycol, 78 mg of 3-(4'-methyl-1-piperazinyl-iminomethyl-^{14}C)-rifamycin SV. with a specific activity of 3.0 μCi/mg, was injected i.v. into the femoral vein of a 12 kg, 6-year-old male Rhesus monkey. Urine and faeces were collected separately in a metabolism cage. 6 h after injection the animal was killed with CO_2, and blood was taken from the femoral vein, together with the 16 tissues shown in the Table.

Segments of both median and sciatic nerve approximately 4–5 cm in length were removed after slitting the epineurium, taking great care to avoid contamination by blood or tissue fluid.

Distribution of radioactivity in monkey tissues 6 h after an i.v. dose of 6.48 mg/kg C^{14}-Rifampicin

Tissue	μg/g[a]	Tissue[a]	μg/g or ml
Left brachial nerve	2.12[b]	Tongue	6.33
Right brachial nerve	1.92	Spleef	6.72
Left sciatic nerve	2.29	Lung	8.32
Right sciatic nerve	2.09	Pancreas	14.76
Brain[c]	0.87	Testes	6.19
Liver[c]	60.41	Salivary gland	12.78
Fat[c]	1.57	Kidney[c]	14.69
Thyroid[c]	6.96	Plasma	4.64
Epididymis	8.14	Blood[c]	8.19
Heart[c]	10.11	Muscle[c]	4.55
Adrenal[c]	11.83		

[a] Concentration calculated using a specific activity of 3.0 μCi/mg, and is given as Rifampicin equivalents, i.e. Rifampicin plus any metabolites formed. [b] Each value is the average of 2, and in the case of blood and plasma 3, samples of tissue. [c] Tissues referred to in the text, the radioactivity in which, together with urine and faeces, accounted for about 80% of the dose administered.

The radioactivity of the tissues was determined by dissolving approximately 200 mg of each tissue in 2 ml 2-propanol: Soluene (Packard Instruments Ltd.) (1:1 v/v) and then diluting the resulting solution with 15 ml Insta-Gel (Packard Instrument Ltd.): HCl (9:1 v/v) for counting. Blood samples (0.5 ml) were decolourized with 0.5 ml H_2O_2 before adding the scintillator. Urine and plasma (both 0.5 ml) were dissolved directly in scintillator. Faeces were dried, powdered, and combusted, the resulting $^{14}CO_2$ collected and measured.

Radioactivity was measured by scintillation counting. Quenching was corrected for by the internal standard method using *n*-(1-^{14}C) hexadecane (Radiochemical Centre, Amersham).

Results are shown in the Table. The highest amount of radioactivity per g of tissue was found in liver and the lowest in nervous tissue and fat. The nerve to blood ratio averaged 0.25 in the 4 nerves examined. Part of the radioactivity in tissues is probably associated with the blood contained within them, but unfortunately we can find no data on the amount of blood contained in the endo- and peripneurial zones of nerve after removal of the epineurium with its comparatively large vessels. However if the value for human medulla[8]. is of relevance then the maximum contribution of radioactivity from blood found in nerve would be approximately the equivalent of 0.02 μg/g i.e. leaving approximately 1.98 μg/g in nerve itself.

Of the total dose, 10.5% was excreted in the urine and 8.3% in the faeces during the experiment. In all about 80% of the dose could be accounted for by that in the excreta plus the total amount found in those tissues marked with[c] (Table).

[1] R. J. W. REES, J. M. H. PEARSON and M. F. R. WATERS, Br. med. J. *1*, 89 (1970).
[2] D. L. LEIKER and H. KAMP, Lepr. Rev. *41*, 25 (1970).
[3] C. C. SHEPARD, L. LEVY and P. FASAL, Am. J. trop. Med. Hyg. *21*, 446 (1972).
[4] C. C. SHEPARD, Int. J. Lep. *40*, 97 (1972).
[5] F. F. WILKINSON, J. GAGO and E. SANTABAYA, Int. J. Lep. *40*, 53 (1972).
[6] J. LANGUILLON, Méd. Afr. Noire *18*, 765 (1971).
[7] M. OZAKI, US-Jap. Coop. med. Sci. Programm. 8th Joint Conference (1973).
[8] W. LIERSE, Acta Anat. *54*, 1 (1963).