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].

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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.

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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).

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The present results confirm penetration of Rifampicin into the substance of peripheral nerve, internal to the epineurial covering. A search of the literature has failed to reveal other studies on the penetration of anti-leprosy drugs into primate nerve. However, a preliminary communication[9], using a chemical method of analysis in the dog and sheep, has indicated good penetration into sciatic nerve, and KEBERLE[10] reported the presence of C^{14}-labelled Rimactane in whole mouse sciatic nerve. Although Rifampicin has a considerable degree of lipid solubility, and has not in fact been reported as excluded from any tissue or body fluid so far analyzed, and has a unique ability to penetrate polymorphs and kill intracellular staphylococci[11], its exact site of penetration into peri- and endo-neurium, and into Schwann cell, myelin and axon of mammalian nerve – either normal or diseased – has still to be established.

In making any comment on likely bactericidal levels of this drug in nerve one must bear in mind that its minimal inhibitory concentrations against the leprosy bacillus in any tissue cannot as yet be determined all that accurately. However, from previous results in blood[12,13], there are grounds for believing (Dr. C. C. SHEPARD, personal communication) that the levels here recorded in nerve may indeed be bactericidal for *M. leprae*.

Summary. The penetration of C^{14} Rifampicin into various tissues, but particularly peripheral nerve, has been studied in the monkey. Penetration into the substance of peripheral nerve internal to the epineurial covering was demonstrated and the significance of this in relation to the treatment of leprosy is discussed.

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Effect of Albumin on Uncoupling of Oxidative Phosphorylation by Chinoform in Rat Liver Mitochondria

Administration of massive doses of chinoform (5-chloro-7-iodo-8-quinolinol) has been considered in Japan to cause a neuropathy, called SMON (*s*ubacute *m*yelo-*o*ptico *n*europathy). The toxicity of this drug has been investigated by a variety of methods. It was found recently in this laboratory that chinoform is an uncoupler of oxidative phosphorylation, and that cations such as magnesium or ferric ions are necessary for this uncoupling action[1]. On the other hand, it was shown that chinoform circulates in the blood as its ablumin complex[2]. This paper deals with the toxicity of chinoform-albumin complex as reflected by its uncoupling action on isolated rat liver mitochondria.

Chinoform was recrystallized from ethanol before use; ADP and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis. Rat liver mitochondria were isolated essentially according to the method of HOGEBOOM[3], using a medium containing 0.21 *M* mannitol, 0.07 *M* sucrose, and 0.1 m*M* EDTA[4]. Three additional washings were performed to reduce the amount of light mitochondria and other contaminants. Mitochondrial protein was determined by the biuret method[5]. Oxygen uptake at 20 °C was measured using a Beckman Oxygen Sensor. The standard reaction mixture (2.5 ml) contained 0.3 *M* mannitol, 10 m*M* KCl, 10 m*M* KH_2PO_4, 5 m*M* *Tris*-HCl (pH 7.4), 2.5 m*M* $MgCl_2$, and 0.25 m*M* EDTA. The reaction was started by the addition of mitochondria (mitochondrial protein, 2 mg/ml) followed by succinate (8 μmoles/ml). Chinoform dissolved in dimethyl sulfoxide was added to the reaction medium. Dimethyl sulfoxide at the concentrations employed had no effect on oxygen uptake. States 3 and 4 of the released respiration are defined according to CHANCE and WILLIAMS[6].

Figure 1-A shows the trace of oxygen consumption during a typical experiment. On addition of ADP (200 nmoles/ml), the respiration showed characteristic state 3-4-3 cycle, ADP/O ratio and respiratory control index (RCI) being 1.9 and 4.2, respectively. On subsequent addition of chinoform (200 nmoles/ml), the state 4 respiration rate reversed to state 3 respiration. As shown in Figure 1-B, this uncoupling action of chinoform was not observed if bovine serum albumin (4 mg/ml) was added prior to the addition of chinoform. This result indicates that either the chinoform-albumin complex is inert as an uncoupler or albumin protects the mitochondria from the uncoupling action of chinoform. To distinguish between these two possibilities, a further amount of chinoform was added. This was found to release the controlled respiration. It was further revealed that 4 mg bovine serum albumin (about 58 nmoles) was required to abolish the uncoupling action of 200 nmoles of chinoform (molar ratio, about 1:4). These results indicate that bovine serum albumin forms a complex with chinoform and the complex is inactive as

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