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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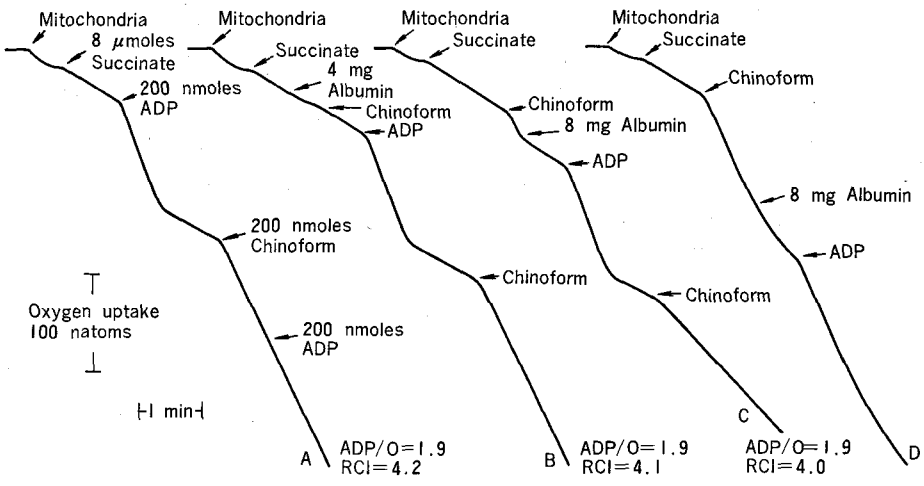

Fig. 1. Effect of albumin on uncoupling action of chinoform on oxidative phosphorylation of rat liver mitochondria. Reaction mixture (2.5 ml) contained 0.3 *M* mannitol, 10 m*M* KCl, 10 m*M* KH_2PO_4, 5 m*M* *Tris*-HCl (pH 7.4), 2.5 m*M* $MgCl_2$, and 0.25 m*M* EDTA. The reaction was started by the addition of mitochondria (mitochondrial protein, 2 mg/ml) followed by succinate (8 μmoles/ml). Further additions were made as shown in the figure. Amounts indicated are per ml of reaction mixture. Reaction temperature was 20 °C. The amounts of additions for B–D are the same as those for A, unless otherwise mentioned.

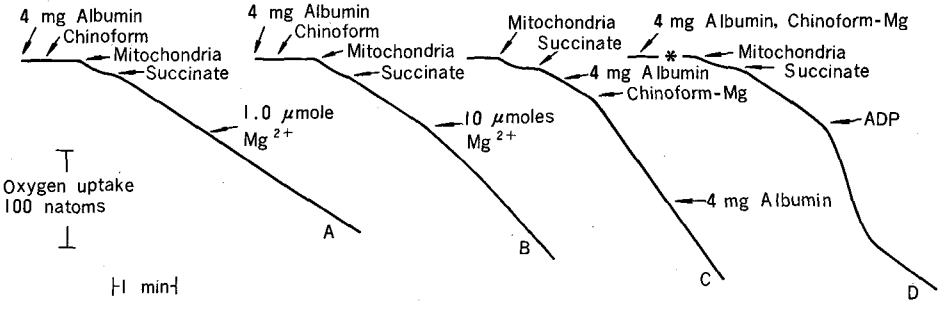

Fig. 2. Interaction between magnesium ions and albumin on uncoupling of oxidative phosphorylation by chinoform in rat liver mitochondria. Reaction mixture and other experimental conditions are the same as those in Figure 1. *Showing the duration of 15 min.

an uncoupler. This observation is similar to that on the effects of other uncoupling agents reported by WEINBACH and GARBUS[7]. Furthermore, it was revealed by the experiment shown in Figure 1-C that the uncoupling action of chinoform can be reversed by the addition of albumin, if albumin was added soon after chinoform. This reversal of inhibition by albumin, however, could not be expected after release of the controlled respiration by chinoform was completed (Figure 1-D). The results represented in Figure 1 could be reproduced in 5 independent experiments without any exception. The beneficial effect of serum albumin on mitochondria was considered to be specific for serum albumin, since it was not observed with lactoalbumin, lactoglobulin and beef haemoglobin.

Since our previous data[1] indicate that the uncoupling action of chinoform requires cations such as magnesium ions, the effect of magnesium ions in the presence of albumin was studied. As shown in Figure 2-A, the chinoform-albumin complex revealed no uncoupling effect upon addition of 1.0 μmole/ml of magnesium ions. However, further increase in magnesium ions provoked the uncoupling action (Figure 2-B). This result suggests that magnesium ions combine with chinoform in competition with albumin to form a chinoform-magnesium chelate that is active as an uncoupler. This view is supported by the experimental data shown in Figure 2-C and D. The uncoupling action of chinoform-magnesium chelate was not diminished by prior addition of albumin. However, when chinoform-magnesium chelate was incubated with albumin for 15 min, the uncoupling action was diminished. Five independent experiments gave results similar to those of Figure 2. These results can be interpreted to mean that albumin combines with chinoform in competition with magnesium ions and the equilibrium of these molecular interactions is established very slowly.

In our previous paper[1], it was reported that the uncoupling effect of chinoform is not observed if magnesium ions are added after chinoform. The present observation that magnesium ions added after the addition of chinoform are effective, should be ascribed to the presence of albumin. It can be reasonably argued that chinoform, added to the reaction mixture in the absence of both magnesium ions and albumin, precipitates in its free form because of its low solubility in the medium, while it forms a chelate or albumin complex in the presence of magnesium ions or albumin. Therefore, it can be concluded that magnesium ions can combine with chinoform solubilized by forming a complex with albumin, but not with precipitated chinoform.

From the present results, it is considered that chinoform absorbed is transferred to tissues in the form of its complex with serum albumin and deposited in tissues by some mechanism, in which chelate formation would be an important event.

Summary. Addition of serum albumin diminished the uncoupling effect of chinoform on oxidative phosphorylation in rat liver mitochondria. Upon increasing the concentration of magnesium ions in the medium, the action of serum albumin was diminished. These results indicate that serum albumin combines with chinoform in competition with magnesium ions.

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