dering that they are quaternary nitrogen compounds with no alcohol-containing side chains at all. This finding indicates that quaternary ammonium compounds have an affinity for the uptake mechanism, and supports our suggestion above that a quaternary ammonium recognition site is a major feature of the transport system. Chlorpromazine and haloperidol, drugs that are known to block dopamine receptors and to have effects on cholinergic systems *in vivo* [12], showed $IC_{50}$ values of 22 and 60 $\mu$M respectively. Thus it appears that a relatively wide variety of drugs and compounds can affect choline uptake. While this does not detract from the usefulness of the three guidelines presented above, it implicates uptake inhibition as some part of the mechanism of action of these various drugs.

While these studies point out some potent uptake inhibitors, we cannot establish, on the basis of these data alone, whether they are transported by the choline system or acetylated. However, acetylation of these compounds by choline acetyltransferase can be examined directly [6, 7], and there are two types of experiments that one can perform to test whether a compound is transported. One approach is to study the kinetics of transport; the similarity of the transport constant ($K_t$) of a compound and its inhibition constant ($K_i$) for choline transport indicates that the same transport system may be involved. Another approach is to show a reduction of uptake of the compound in question in synaptosomal preparations after cholinergic nerve terminals have degenerated [4]. By utilizing these procedures, it has recently been shown that monoethylcholine and pyrrolidine choline are transported by this system [6, 7], but acetylcholine is not [11]. Additional studies have shown that monoethylcholine, diethylcholine, triethylcholine and pyrrolidine choline (potent uptake inhibitors) are transported, acetylated, and in some cases form false neurotransmitters [6, 7, 9, 10]. Thus, the data presented here provide one with a framework for designing compounds which may be potent inhibitors of choline uptake, or compounds that would be transported by cholinergic nerve terminals and perhaps form false neurotransmitters.

A preliminary report of this study has been presented [13].

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# Effect of androgenic steroid nitrates on rat liver lysosomes *in vitro*

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It is generally accepted that anti-inflammatory steroids stabilize the lysosomal membrane. This effect might be one of the mechanisms of their anti-inflammatory action as shown by studies *in vitro* of Weissman *et al.* [1–3] and by experiments *in vivo* [4, 5]. However, Tanaka and Iizuka [6] showed that the purified "light" lysosomal fraction, unlike the crude lysosomal fraction, is not stabilized by anti-inflammatory drugs. Malbica [7] and Malbica and Hart [8] working with a purified lysosomal fraction at pH 7·4 could not find any stabilizing effect of hydrocortisone and cloroquine.

In previous experiments we found that some androgenic steroid nitrates (4-chloro-testosterone nitrate and androstanolone nitrate) have anti-inflammatory activity against exudative experimental inflammation (formaline, kaolin and carrageenin edema and cotton pellet inflammation), whereas another compound from the same class, testosterone nitrate, has pro-inflammatory activity. Thus, it seems worthwhile to study their effect on lysosomes, attempting to find a correlation with their action on the experimental inflammation.

The effect of incubating the particulate fraction containing mitochondria and lysosomes (ML) isolated from rat liver [9] with steroid nitrates, synthesized according to published procedures [10] and with one of the parent compounds, androstanolone, for 90 min at 37° and pH

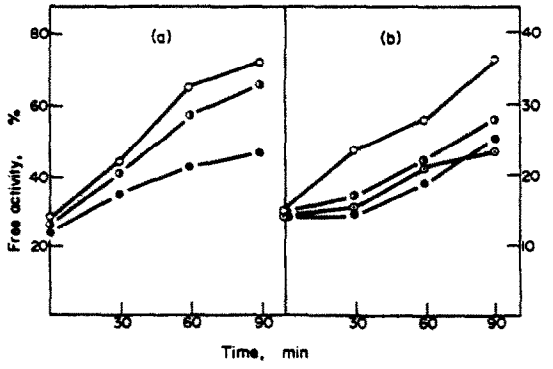


Fig. 1. Time-dependent changes in "free" acid phosphatase (a) and β-glucuronidase (b) activity of ML fraction incubated at pH 7·4 with androstanolone, androstanolone nitrate and cholesterol. Experimental conditions were the same as described in Table 1, with 12·2 mg of protein/ml. Final concentration of androstanolone (⊙), androstanolone nitrate (●) and cholesterol (◑) in ethanolic solutions was $10^{-4}$ M. Reference sample (○) contained 0·01 ml ethanol.

7·4 is illustrated in Table 1. The three anti-inflammatory steroids have a protective effect on the lysosomes, which is not observed with testosterone nitrate. The protective effect of androstanolone nitrate is even more efficient than that of cholesterol used for comparison (Fig. 1a and b). Cholesterol is known as a potent stabilizing agent of lysosomal enzymes [11]. Both the stabilizing effect of androstanolone nitrate and the labilizing effect of testosterone nitrate can be better demonstrated by incubating the particulate fraction for 90 min at 37° and pH 5 (Fig. 2). The actions of other steroids, and even the labilizing effect of testosterone under the same incubation conditions, have been demonstrated previously [12].

The action of steroid nitrates on lysosomes was also tested by exposing the organelles to media containing different concentrations of sucrose, and by measuring in these conditions the release of the "free" β-glucuronidase activity. As shown in Fig. 3, both the protective effect of androstanolone nitrate and the labilizing action of testosterone nitrate can be observed.

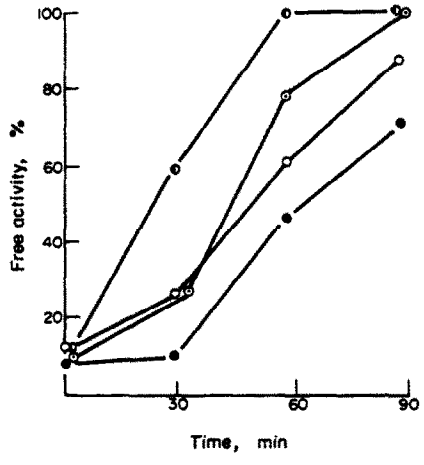


Fig. 2. Time-dependent change in "free" β-glucuronidase activity of ML fraction incubated at pH 5 with androgenic steroid nitrates. 1 ml of ML fraction (10·9 mg of protein) suspended in 0·25 M sucrose buffered with 25 mM acetate-acetic acid, pH 5, was incubated at 37° with $10^{-4}$ M androstanolone nitrate (●), $10^{-4}$ M testosterone nitrate (⊙) and 2 × $10^{-4}$ M testosterone nitrate (◐) in ethanolic solutions. Reference sample (○) contained ethanol. At 30, 60 and 90 min, samples were taken for β-glucuronidase assay.

Table 1. Effect of steroid nitrates *in vitro* on free activity of β-glucuronidase and acid phosphatase from ML fraction of rat liver

| Addition | β-Glucuronidase $10^{-5}$ M | β-Glucuronidase $10^{-4}$ M | Acid phosphatase $10^{-5}$ M | Acid phosphatase $10^{-4}$ M |
|---|---|---|---|---|
| Control | 34·6 | | 58·0 | |
| Androstanolone | 28·3 | 25·8 | 37·6 | 33·0 |
| Androstanolone nitrate | 25·4 | 14·5 | 48·9 | 44·0 |
| 4-Chloro-testosterone nitrate | 32·2 | 30·7 | 57·0 | 45·1 |
| Testosterone nitrate | 34·2 | 36·8 | 57·5 | 58·1 |

1 ml of ML fraction (10 mg of protein) suspended in 0·25 M sucrose buffered with 10 mM Tris–HCl, pH 7·4, was incubated at 37° for 90 min with 0·01 ml steroid nitrates in ethanolic solutions, so that their final concentration was $10^{-5}$ and $10^{-4}$ M. The reference sample contained 0·01 ml ethanol. Thereafter the enzyme activity was measured by colorimetric determination of phenolphthalein released from phenolphthalein-β-monoglucuronide and of inorganic phosphorus released from Na β-glycerophosphate at 37° and pH 5 [9]. Free activity is expressed as per cent of activity before and after treatment of organelles with lubrol XW (β-glucuronidase) or four successive freeze–thawings (acid phosphatase).

The results show that the androgenic steroid nitrates have both a stabilizing and labilizing action on the rat liver lysosomes, without any effect on the "total" activity of lysosomal enzymes, according to their effect on the experimental inflammation *in vivo*. However, this conclusion is only qualitative, due to the limits of this kind of experiment; these limits could even explain some of the contradictory data mentioned in the introduction. Since the lysosomes are structurally and functionally heterogenous [13, 14] the effect of different agents acting on

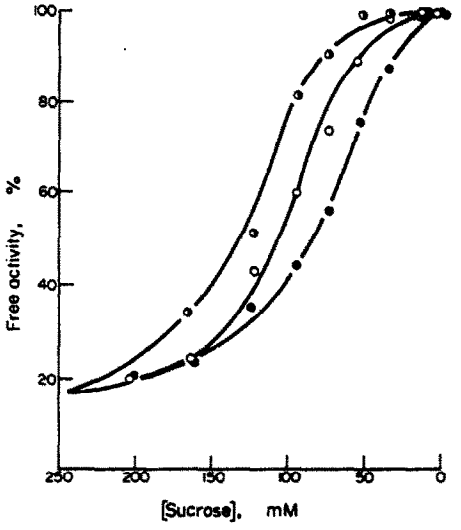


Fig. 3. Effect of androstanolone nitrate and testosterone nitrate on osmotic resistance of rat liver ML fraction. 1 ml of ML fraction (14 mg of protein) was incubated for 20 min at 0° with 0·01 ml androstanolone nitrate (●) or testosterone nitrate (◑) in ethanol, their final concentration being $10^{-4}$ M. The reference sample (○) contained 0·01 ml ethanol. Each sample was used to make eight diluted solutions in sucrose with molarities between 0·012 and 0·25 M. After incubation of these diluted samples for 10 min at 0°, β-glucuronidase activity was assayed at 37° as previously described [9]. The results are expressed as per cent of β-glucuronidase activity from the activity of organelles previously treated with lubrol XW.

them is likely to vary. Consequently we have to consider the statistical behaviour of these heterogenous population as shown by "free" activity distribution curves shown in Fig. 3.

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