frequency of the fibre diameters was about 30 μm in treated mice, while in controls it was about 50 μm (figure 2).

The protein content of the gastrocnemius muscle in treated mice was significantly lower in comparison with the control group: this reduction had its counterpart in the markedly increased hydroxyproline content (table). The above mentioned data permit one to qualify the lesion as a muscular dystrophy, although a clear-cut distinction of its origin is impossible, since in this field the pathological findings are confusingly similar, and morphology depends on the stage that the disease has reached in the particular muscle examined. The morphological data fit very well with the biochemical findings and the functional tests on muscle.

	Vehicle	Δ^9-THC
Weight of the muscle (mg)	171.7 ± 7.89*	187.2 ± 12.2
Protein content (per cent of the weight of the muscle)	19.72 ± 0.58	15.57 ± 0.35 ($p<1^0/_{00}$)
Hydroxyproline content (per cent of the protein content)	0.637 ± 0.03	1.065 ± 0.05 ($p<1^0/_{00}$)
Twitch tension (g)	59.85 ± 2.33	36.70 ± 2.26 ($p<1^0/_{00}$)
Tetanus tension (g)	218.86 ± 8.87	160.28 ± 8.33 ($p<1^0/_{00}$)

* Standard error.

It can therefore be inferred that the treatment with Δ^9-THC at low doses for a rather prolonged time gives rise to a particular muscular lesion which, as far as we know, has never been described after cannabinoids. Our experimental data cannot give an explanation of the possible mechanism involved in the origin of this peculiar dystrophy. However, the data reported by Kayaalp et al.[7] account for a partial blocking action of Δ^9-THC upon neuromuscular transmission, possibly due to a direct action of the drug on the naked motor nerve terminals in the muscle, which are more susceptible to such an action than the myelinated motor axons in the nerve trunk. Therefore, it cannot be excluded that such a blocking action, protracted for 30 days, is one of the causes of the observed muscular lesions, though some effects obtained after treatment with Δ^9-THC[2,3,8–10] suggest that a more general mechanism is involved. Studies are in progress on this topic.

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Cardiotonic activities of some new type of bufadienolide- and cardenolide-conjugates[1]

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Summary. Some new type of bufadienolide- and cardenolide-conjugates, including bufotoxins and 3-sulfates recently isolated from the toad skin, were tested for their cardiotonic activities by using isolated frog hearts and guinea-pig atria. The relative potencies were obtained and reported.

Recently, Nambara and his co-workers[3–11] have isolated new bufotoxins and their homologs, as well as bufadienolide and cardenolide 3-sulfates, from the skin of Japanese toads. They also synthesized the analogous conjugates of cardenolide[12,13]. In this study, some of these compounds were tested: 1. the isolated frog hearts, and 2. the isolated guinea-pig atria.

The agents used were bufalin 3-sulfate, digitoxigenin 3-sulfate*, gamabufotalitoxin (gamabufotalin 3-suberoyl-arginine ester), gamabufotalin 3-hemisuberate, gamabufotalin, 8 digitoxigenin 3-suberoyl-X esters* in which X were amino acids, and dipeptides (table 2), as well as bufalin and digitoxigenin (* = synthetic specimen).

The stock solution of digitoxigenin was prepared with 95% ethanol in concentration of 1.0 mg/ml (2.7 mM). All other compounds except 3 were also dissolved in concentration of 1.0 mg/ml. Stock solutions of gamabufotalitoxin, gamabufotalin 3-hemisuberate and gamabufotalin were made in equimolar to that of digitoxigenin. Immediately before experiment, these stock solutions were diluted with saline (0.6% for frog hearts, and 0.9% for guinea-pig atria) to desired concentrations.

1. The isolated frog heart (Straub's preparation). The method of assay is the same as the previous paper[14]. Male frogs, Rana nigromaculata (20–35 g) were used. The Straub's cannula contained 2 ml of Ringer's solution, the

1 This study was reported at the 49th General Meeting of the Japanese Pharmacological Society, 31 March 1976, Osaka.
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composition of which (in mM) was: NaCl 111, KCl 2.7, $CaCl_2$ 1.8, $NaHCO_3$ 15 and glucose 2.7. It was aerated with 95% O_2 + 5% CO_2. Isotonic contraction of the heart was recorded on smoked drums. The heart was first made hypodynamic by reducing the concentration of calcium to 0.6 mM, $^1/_3$ of the normal, and the effects of the compounds were tested in the following way.

Starting from a subthreshold dose, a small amount (10–70 µl) of a diluted solution was added to the cannula every 15–30 min, so that a stepwise increase in the cumulative concentration of the test compound was achieved, until the heart went into contracture. The way of increasing the cumulative concentration was: 10^{-n}, 3×10^{-n}, $10^{-(n-1)}$,... A next addition was not made until the height of contraction reached a plateau. The relative potencies (RP) were obtained on the basis of the concentration of each compound in which contracture of the heart was brought about (C-contr). Each compound was tested on 4 preparations. The experiments were carried out with several lots of frogs at room temperature of 20–25 °C. The potency of digitoxigenin in the same lot of animals was taken as standard. In most cases, C-contr of digitoxigenin was 3×10^{-7} g/ml. Occasionally it was 10^{-6} g/ml (1 out of 5–6 cases). In the following, the RP-values are shown on molar basis.

Table 1. Relative potencies of compounds belonging to group A and group B obtained in frog hearts and guinea-pig atria

Compound	RP Frog heart	Guinea-pig atria Con-tracture	SQ
A.			
Digitoxigenin	1.0	1.0	1.0
Digitoxigenin 3-sulfate	0.3		
Bufalin	0.3	10	10–30
Bufalin 3-sulfate	1.0		
B.			
Digitoxigenin 3-suberoyl-arginine ester	0.3–1.0	0.3	0.3
Gamabufotalin	1.0	3.0	3.0–10
Gamabufotalin 3-hemisuberate	0.3	1.0	1.0
Gamabufotalitoxin	1.0	1.0–3.0	3.0

RP = relative potency. SQ = sudden quickening (see text). Dixitoxigenin was taken as standard. RP's are shown on molar basis.

Table 2. Relative potencies of compounds belonging to group C obtained in frog hearts.

C. Digitoxigenin 3-suberoyl-X esters X	RP
1. Glycine	0.3–1.0
2. L-Alanine	0.1–0.3
3. L-Tryptophan	0.3
4. L-Proline	0.03
5. L-Arginine	0.3–1.0
6. Glycyl-L-proline	0.1–0.3
7. L-Aspartic acid dimethyl ester	0.3
8. Glycyl-L-phenylalanine methyl ester	0.3–1.0

RP = relative potency. Digitoxigenin was taken as standard (1.0). RP's are shown on molar basis.

A. Bufalin 3-sulfate and related compounds: The results are shown in table 1. The RP's of digitoxigenin, bufalin and their sulfates were within a narrow range, 0.3–1.0. Sulfation at C-3 decreased the potency in digitoxigenin, while it increased the potency in bufalin.

B. Gamabufotalitoxin and related compounds: As shown in table 1, the RP's of gamabufotalitoxin and 3 related compounds ranged 0.3–1.0.

C. Digitoxigenin 3-suberoyl-X esters: The RP's of 8 compounds of this group are summarized in table 2. They also ranged within a relatively narrow range, 0.1–1.0, except suberoylproline ester, the potency of which was exceedingly low. Any simple structure-activity relationship was not obtained. The suberoylarginine ester is also included in this table.

The patterns of response of the Straub's preparation to the compounds described were basically the same. The cumulative application caused a gradual increase in the height of contraction, and then a contracture, which was accompanied, or soon followed, by an arrest. The only difference noted in the responses to gamabufotalitoxin and the compounds belonging to the group C was the fact that a longer time (20–30 min) was necessary to reach a steady level of contraction, in every step of the cumulative addition of the drug. In digitoxigenin or the bufogenins, a plateau was reached within 15 min or less.

The heart rate ranged between 40 and 70/min when the preparations were set up. No consistent or remarkable change was caused either by reducing the calcium concentration of Ringer's solution, or by the cumulative addition of the drugs until the concentration reached one or two steps prior to the final (C-contr). Then the beating rate dropped quickly, and an arrest followed.

2. The isolated guinea-pig atria. Male guinea-pigs (370 to 570 g) were used. The right and left atrial preparations were prepared from the excised heart, and suspended separately in organ baths which contained 10 ml of Krebs-bicarbonate solution. The composition of the solution (in mM) was: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.18, $NaHCO_3$ 24.9, and glucose 11.1. It was aerated with 95% O_2 + 5% CO_2 and kept at 37 °C. The contractile tension of the atria was recorded isometrically on an ink-writing oscillograph with a force-displacement transducer and a carrier amplifier. The left atrial preparation was stimulated at a constant rate (4 Hz) by a square-wave pulse. The right beat spontaneously, and the beat rate was recorded with a cardiotachometer. Administration of the drugs was made in a cumulative manner. Digitoxigenin, bufalin, digitoxigenin 3-suberoylarginine ester, gamabufotalin and its 3-hemisuberate, and gamabufotalitoxin were tested. Four experiments were carried out for each compound.

When the concentration of the compound tested was elevated cumulatively, the contractile force of the left atrium increased gradually and reached a peak. Then arrhythmic contractions which did not follow the electrical stimulation appeared, and the systolic tension tended to decrease slightly. The latter was accompanied by a marked increase in the diastolic tension, and a contracture followed. In the right atrial preparation, the inotropic response was almost the same as in the left. A sudden increase in the beat rate (sudden quickening, SQ) invariably preceded the contracture. Digitoxigenin caused contracture and SQ at a concentration of 10^{-6} g/ml. Taking this as standard, the RP's of other compounds were obtained (on molar basis) and summarized in table 1. As shown in the table, the values of the 2 sets of RP's, calculated on the basis of C-contr or the concentration at which SQ appeared, were about the same.

It is well known that bufadienolides are several times more potent than the cardenolide homologs[15]. This relationship is seen in guinea-pig atria in RP-values for bufalin and digitoxigenin, gamabufotalitoxin and digitoxigenin 3-suberoylarginine ester. The same relation, however, was not found in frog hearts. Since the sensitivity of frog hearts to digitoxigenin is about the same as that of guinea-pig atria, the RP's for the 4 bufadienolide-compounds are clearly larger in guinea-pig atria than in frog hearts. This suggests that the frog heart is less sensitive to bufadienolides, but not to cardenolides, than the mammalian heart.

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α- and β-activity of O-methylated derivatives of norepinephrine and epinephrine

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Summary. Metanephrine, iso-metanephrine, normetanephrine and isonormetanephrine were tested for α- and β-activity on various tissues obtained from rats, guinea-pigs and cats. It was found that methylation of the hydroxyl groups of norepinephrine or epinephrine in either the 3- or 4-position markedly reduces or abolishes α- and β-activity with the exception of the nictitating membrane of the cat. This receptor seems to show a tissue difference.

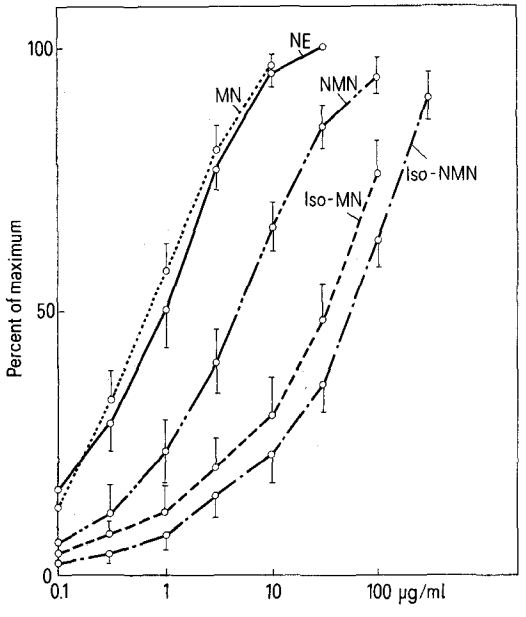
O-Methylation of norepinephrine or epinephrine is generally regarded as an inactivation process and the metabolites metanephrine and normetanephrine are thought to possess little or no activity on α- or β-receptors[2-4]. In contrast, Langer and Rubio[5] found recently that normetanephrine and metanephrine elicited responses of the cat's nictitating membrane equal to that of norepinephrine.

Since it is possible that this discrepancy is the result of a species and/or tissue difference, metanephrine and normetanephrine were tested on a variety of tissues obtained from rats, guinea-pigs and cats. In addition, the 2 isomers of metanephrine and normetanephrine, iso-metanephrine or 3-hydroxy-4-methoxy-N-methyl-phenylethanolamine and iso-normetanephrine or 3-hydroxy-4-methoxyphenylethanolamine, which had not been tested previously, were included in the experiments to obtain information on the importance of the methoxy-group in the 3- and 4-position.

Cats were anesthetized with ether to obtain the nictitating membrane or were sacrificed with an overdose of pentobarbital to obtain vas deferens and spleen. Rats and guinea-pigs were stunned with a blow to the head and quickly decapitated. The testing was performed with conventional methods and the experimental procedures have been described in detail[6-8]. Cumulative dose-response curves were obtained for auricles and nictitating membranes whereas all the other tests were performed by intermittent administration of the test compounds.

The figure shows the dose-response curves of the derivatives on the nictitating membrane of the cat. All compounds are active and the curves are practically parallel. Iso-metanephrine and iso-normetanephrine were less potent than the corresponding isomers.

The table shows a summary of the tissues tested. With the exception of the nictitating membrane, all other tissues did not respond or responded by less than 20% of the maximum response to norepinephrine even when the highest concentrations of the derivative were used.



Effect of O-methylated derivatives of norepinephrine and epinephrine on the nictitating membrane of the cat. The number of determinations was 6 in most instances. The curve for norepinephrine included for purposes of comparison is obtained from Trendelenburg et al.[7].

1 Acknowledgment. W. H. V. would like to thank the Federal Republik of Germany for the "U. S. Senior Scientist Award' which enabled him to work at the Department of Pharmacology, University of Mainz, and to conduct part of this work. W. H. V. would also like to thank Prof. Dr E. Muscholl, Mainz, for his hospitality, help and advice during this stay.

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