the tissues. The hypothesis of activation of the uncoupling factor in the course of mitosis is confirmed by stimulation of respiration of sea urchin and amphibian eggs, which has been found to reach a maximum in the prophase of mitosis [8, 12], and also by information on the fall in the ATP concentration in the regenerating rat liver at the time of maximal mitotic activity [1].

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DETECTION OF RESERPINE-LIKE SUBSTANCES IN THE MYOCARDIUM OF ANIMALS AND MAN

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Substances similar in some of their physicochemical properties to reserpine were found in the myocardium of animals and man. The tissues of the myocardium were shown to synthesize these substances from formate and tryptophan.

KEY WORDS: *myocardium; biosynthesis of reserpine-like substances.*

Alkaloids are a group of organic compounds, as a rule of plant origin, with considerable and varied biological activity. At the beginning of the 1970s the first reports appeared of the discovery of alkaloids in animal tissues [1-3] and the possibility of their biosynthesis in the tissues of mammals by condensation of L-dopa and two of its mono-O-methyl esters with formaldehyde and acetaldehyde [2].

These investigations led the present writers to look for the presence of alkaloid-like compounds in the tissues of animals and man and, in particular, in the myocardium.

EXPERIMENTAL METHOD

The substances for study were isolated from the myocardium of the right and left ventricles of man, ox, and Wistar rats. The human heart was used not later than 12 h after death;

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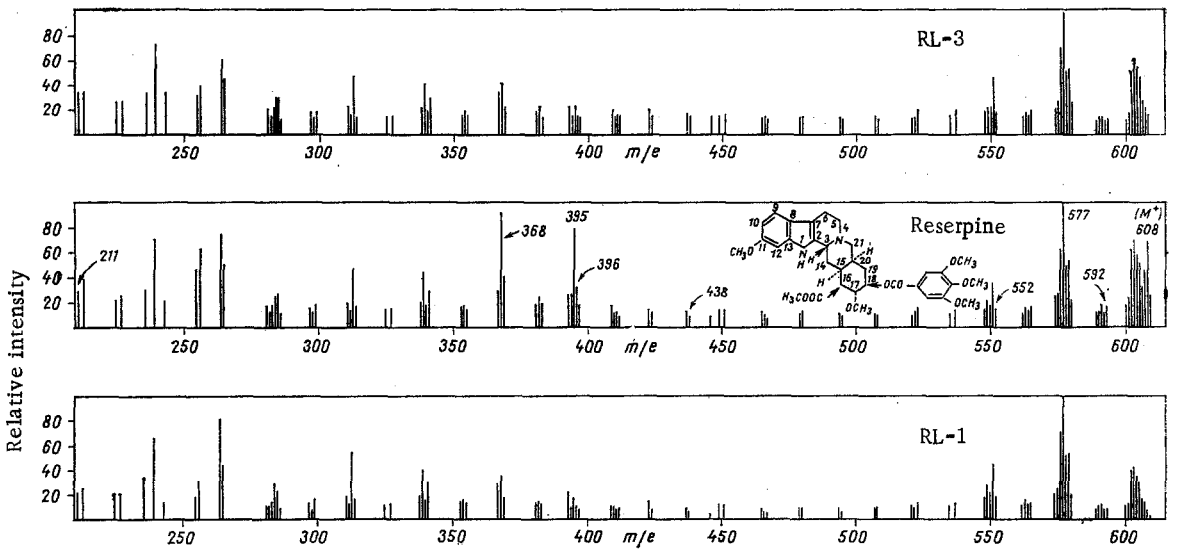
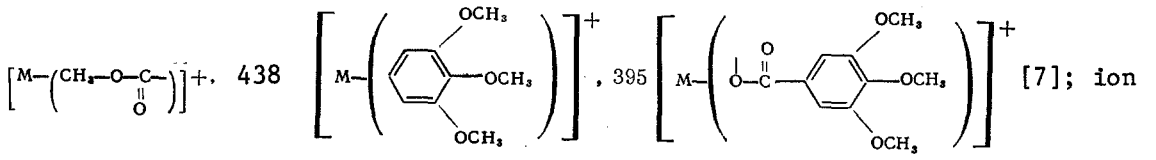


Fig. 1. Mass spectra of RL-1, RL-3, and reserpine. Ionization energy 70 eV. Temperature 150°C. Ions with m/e 608 (M^+), 592 $(M - CH_3)^+$, 577 $(M - OCH_3)^+$, 552



with m/e 211 is trimethoxybenzoic acid.

TABLE 1. Mobility R_f of Alkaloid-Like Fractions in Various Chromatographic Systems

Fractions	Chromatographic systems						
	n-butanol–acetic acid–water (4:1:1)	n-butanol–ethylacetate–water (6:2:2)	acetone–carbon tetrachloride–iso-octane–petroleum ether (7:4:3:6)	chloroform–methanol (95:5)	n-butanol–methylethylketone–water (65:25:25)	methanol–methylethylketone–n-heptane (8.4:33.6:58)	isobutanol–acetic acid–water (4:1:1)
RL-1	0,67	0,78	0,0	0,12	0,78	0,28	0,75
RL-2	0,61	0,61	0,0	0,16	0,70	0,20	0,61
RL-3	0,50	0,44	0,0	0,11	0,53	0,14	0,45
Reserpine	0,58	0,38	0,0	0,10	0,49	0,0	0,50

the hearts of the animals immediately after slaughter or (in some cases) after homogenates of myocardium had been kept at −20°C for 1-7 days. The chromatographic analyses were carried out on "Silufol" plates (Kavaler, Czechoslovakia) without a fluorescent indicator. Absorption and fluorescence spectra were photographed on the Perkin-Elmer 402 instrument and the Aminco-Bowman spectrophotofluorometer. Mass spectra were photographed on the LKB-9000 instrument, and the test substances were introduced directly into the ion source. Reserpine from Koch-Light Laboratories was used as the standard. The coefficients of identity of the mass spectra were calculated [4]. Radioactivity was measured on the Mark 2 counter.

EXPERIMENTAL RESULTS

The method used to isolate and purify alkaloid-like compounds from the myocardium was as follows. Myocardial tissue (200-300 g) was cut into small pieces and ground in 0.4 N $HClO_4$ with sand, previously washed by boiling for 12 h in 6 N HCl. The volume of the paste-like homogenate was made up to 1 liter and its temperature was kept constant at 37°C for 18 h. The filtrate of the homogenate was alkalified with ammonia and extracted with a mixture of chloroform, isobutanol, and n-propanol (13:1:1). The aqueous and organic phases were separated by centrifugation. The organic phase was concentrated in vacuo at 40°C and fraction-

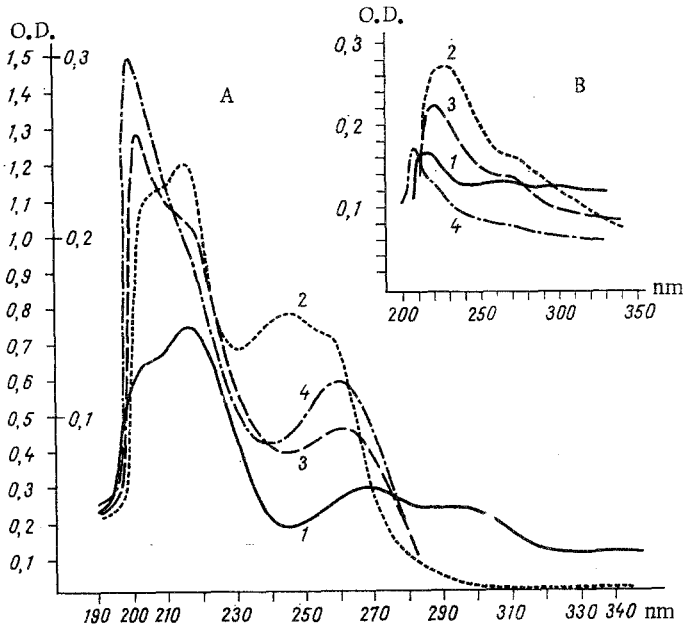


Fig. 2. Absorption spectra of RL-1, RL-2, RL-3, and reserpine. Solvent: A) methanol; B) hexane. 1) Reserpine, scale 1.5 in A and 0.3 in B; 2) RL-1, scale 1.5 in A and 0.3 in B; 3) RL-2, scale 0.3; 4) RL-3, scale 0.3.

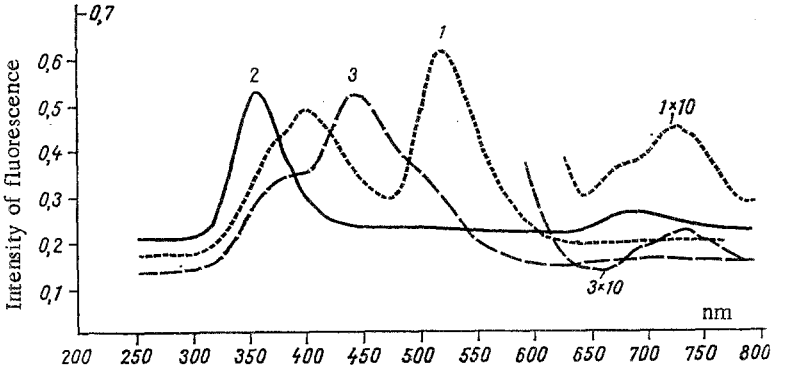


Fig. 3. Fluorescence spectra of RL-1, RL-3, and reserpine. Solvent chloroform. Excitation wavelength 270 nm. 1) RL-3, scale 1.0; 2) reserpine, scale 1.0; 3) RL-1, scale 1.0. For 1 × 10 and 3 × 10, scale 0.1.

ated on "Silufol" plates, using systems of chloroform–methanol (95:5) and isobutanol–acetic acid–water (4:1:1) consecutively. Zones fluorescent in UV light were eluted with a mixture of chloroform, isobutanol, and n-propanol (13:1:1) and rechromatographed in the same system. Ultimately three fractions were isolated (RL-1, RL-2, RL-3). On "Silufol" plates in UV light they gave blue (RL-1) and yellow (RL-2, RL-3) fluorescence. Substances RL-1, RL-2, and RL-3 were precipitated from the solution of dilute perchloric acid by specific reagents for alkaloids: Scheibler's reagent (with phosphotungstic acid) and Wagner's reagent (with iodine and potassium iodine).

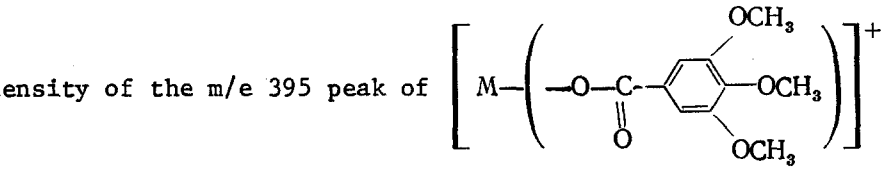
The mobility of the fractions in seven chromatographic systems varied within wide limits (R_f from 0.0 to 0.8), but did not coincide with that of reserpine (Table 1). However, mass-spectrometric analysis of RL-1 and RL-3 and also of reserpine over the range of ions from m/e 12 to m/e 608 showed them to be identical (Fig. 1). The coefficient of identity relative to reserpine was 0.87 for RL-1 and 0.88 for RL-3. Disregarding ions of low specificity, from

m/e 12 to m/e 100, the coefficient of identity amounted to 0.97 and 0.95, respectively. A marked difference was observed only in the intensity of the two ions with m/e 368 and m/e 395, the peaks for which in the mass spectrum of reserpine were about twice as high as in the mass spectra of RL-1 and RL-3.

The absorption spectra for RL-1, RL-2, and RL-3 in the UV region also resemble that of reserpine in several features. In methanol both reserpine and RL-1, RL-2, and RL-3 have two principal characteristic absorption bands at 220 and 270 nm typical of indole-containing compounds, such as reserpine. A common feature of the substances compared is a change in the character of absorption on their transfer from a polar solvent (in this case, methanol) to a nonpolar solvent, such as hexane (Fig. 2).

The fluorescent spectrum of reserpine (Fig. 3) with an excitation wavelength of 270 nm is characterized by the presence of one strong and two weak maxima of fluorescence, at 357, 500, and 690 nm, respectively. The spectrum of RL-1 under the same conditions has two shoulders at 365 and 500 nm and two maxima — strong at 445 nm and weak at 735 nm — whereas the spectrum of RL-3 has two strong peaks at 400 and 520 nm and a weak peak at 735 nm. The fluorescent spectra of RL-1, RL-3, and reserpine thus differ essentially.

These observations suggest that the structures of the RL-1, RL-2, and RL-3 molecules, on the one hand, and the structure of the reserpine molecule, on the other hand, have essential features of similarity but are not identical. The similarity may be that RL-1, RL-2, and RL-3 are polymers or isomers of reserpine (or a mixture of both). Both these derivatives of reserpine have been obtained previously by chemical methods [5, 6]. It does not contradict logic to suggest that the substances in this case are polymers of isomers of reserpine or of its oxidized product. However, it is difficult to imagine that polymers of reserpine and native reserpine would have virtually identical mass spectra, as was observed in these experiments for RL-1, RL-3, and reserpine. A more likely explanation is that the substances studied are associates of 2, 3, 4 or more molecules of reserpine (or of its isomers), linked by a hydrogen bond between the nitrogen atom of one molecule and the NH group of the other. Under these conditions the monomers would preserve the structure appropriate to native reserpine (or its isomers) and also its ability to dissociate readily under electron bombardment in the mass spectrometer, with the formation of a molecular ion (M^+) 608 and other characteristic ions.

The difference in intensity of the m/e 395 peak of  for

RL-1, RL-3, and reserpine confirms the hypothesis that the test substances are isomers, for C_{18}, to which the trimethoxybenzoic acid residue is attached in the reserpine molecule, is asymmetrical. RL-1, RL-2, and RL-3 are possibly associates of one or other form of reserpine with short chains of polypeptides, polynucleotides, or polysaccharides. This is indirectly confirmed by the sharp difference between the fluorescence spectra of reserpine and of the test substances, which can be attributed either to significant conformational differences or to the presence of additional chromophores in the composition of the reserpine-like substances. These latter groups may also determine the differences in fluorescence of RL-1 and RL-3. If this is so, it can be predicted that these additional chromophores are linked by labile bonds with the reserpine-like "nucleus," for ions with higher m/e values than the ion with the molecular weight of reserpine, found in the mass spectra of the test substances, account for only 0.07% of the total intensity of all the other ions. Such "heavy" compounds evidently dissociate almost completely under the high temperature conditions (up to 150°C) in the mass spectrometer even before electron bombardment.

The problem of whether RL-1, RL-2, and RL-3 enter the body from outside or are synthesized by the animal cell can be resolved by the use of radioactive precursors of these compounds. Sodium [^{14}C]formate and [^{3}H]tryptophan were used for this purpose. These compounds were found to be incorporated in vitro into RL-1, RL-2, and RL-3 in homogenates of rat myocardium at 37°C. The radioactivity was determined by transferring scrapings of purified fractions from the chromatograms into flasks containing scintillator (xylol, 0.3% PPO). After incubation for 3 h with [^{14}C]formate the radioactivity of RL-1, RL-2, and RL-3 in one typical experiment was 634, 381, and 69 cpm (homogenate of the myocardium of five rats, 40 μCi) and with

[^{3}H]tryptophan it was 867, 154, and 83 cpm, respectively (homogenate of myocardium of five rats, 5 μCi). [^{14}C]Formate was incorporated into RL-1, RL-2, and RL-3 in vivo also (331, 36, and 230 cpm, respectively; five rats, 200 μCi intraperitoneally, 18 h).

Consequently, the animal cell had an autonomous system for the biosynthesis of RL-1, RL-2, and RL-3.

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RESPONSE OF DIFFERENT TYPES OF CONNECTIVE TISSUE TO HORMONES

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A comparative study was made of the lysosomal glycosidases of the eye tissues (sclera and cornea) and also of bone tissue and cartilage from rabbits. Intraperitoneal injection of thyrocalcitonin (TCT), deoxycorticosterone (DOC), hydrocortisone (HC), and somatotropic hormone (STH) were shown to modify the activity of β-galactosidase, β-glucosidase, and hyaluronidase and the functional state of the lysosomal membranes in the tissues. HC and STH stabilize, whereas DOC and large doses of TCT labilize the lysosomal membranes. After injection of HC and STH the absolute activity of the enzymes in the tissue homogenates falls, whereas DOC has the opposite action.

KEY WORDS: *eye; bone; cartilage; lysosomal enzymes; hormones.*

Changes in the organ of vision are among the signs of various diseases of the skeletal system. Blue sclerotics are found in patients with osteogenesis imperfecta [1]; pathology of the organ of vision is characteristic of Marfan's syndrome, homocystinuria [2], mucopolysaccharidoses [14, 18], etc. Many pathological states of the organ of vision are evidently connected with changes in the activity and location of enzymes and also with disturbances of the functional state of the membranes of the lysosomes. High sensitivity of lysosomal enzymes of carbohydrate metabolism of the connective tissues of the eye (sclera, cornea) to the action of hormones has repeatedly been demonstrated [3, 4].

In this investigation the activity of the lysosomal enzymes of different types of connective tissue and its changes under the influence of certain hormones were compared.

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