evidence that glycine itself was a major source of the formate. Siekewitz and Greenberg (*5*) confirmed Sakami's work and also showed that the liver uptake of methyl-labeled glycine was 1½ times as fast as that of carboxyl-labeled glycine.

The results of this experiment show that the intact liver of mice handles parenterally administered glycine as it does *in vitro*. It is of interest that even the grossly damaged liver of depleted animals behaves similarly, although the conversion is slower and there is the suggestion of an additional metabolic path in the production of radiocystine.

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Identification of Murexine as β-[Imidazolyl-(4)]-Acryl-Choline

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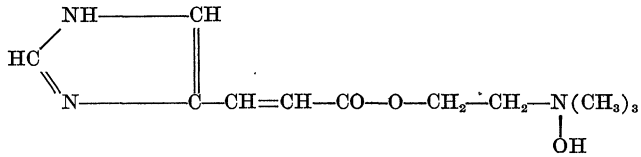
Murexine is an active product contained, often in very large quantities, in the median zone of the hypobranchial body of *Murex trunculus* and of other related species of mollusks. Biologically, it manifests intense nicotinic and curariform actions; chemically, it is a choline derivative.

We had already come to these conclusions some years ago, following our first researches carried out on various pure salts of murexine (picrate, picrolonate, flavianate, styphnate, and reineckate) (*1, 2*).

Further investigations, a preliminary account of which is given in the present paper, have enabled us to clear up the chemical constitution of murexine, and consequently to identify this substance as β-[imidazolyl-(4)]-acryl-choline or urocanyl-choline.



Murexine
(Urocanyl-choline)

Murexine picrate, the starting material of all our researches, is urocanylcholine dipicrate (UCD).

Characteristics of murexine picrate: More or less elongated plates and thin crossed needles, slightly soluble in cold water (less than 0.1%), much more so in boiling water, from which the product may be easily recrystallized; mp 218°–221° C, with decomposition.

(UCD) $C_{11}H_{18}O_2N_3 \cdot C_6H_3O_7N_3 \cdot C_6H_2O_7N_3$ (681)	Calc:	C 40.53;	H 3.35; N 18.50%
Murexine picrate (Sample 1)	Found:	40.68	3.57 18.34%
" " (Sample 2)	"	40.55	3.60 18.71%
" " (Sample 3)	"	40.51	3.60 18.70%

Percentage of picric acid in murexine picrate was determined quantitatively by direct weighing, and by the nitron method according to Busch (*3*). In the direct weighing method, aliquots of 1–4 g of murexine picrate were treated in a boiling water bath with 30–100 ml of 1 *N* HCl for 1–2 hr. After the product was completely dissolved, the solution was cooled and extracted with ethyl ether in a Palkin automatic extraction apparatus, until the solvent removed no more picric acid, and the liquid in the extractor tube had become colorless. The intensely yellow-colored ether in the extraction flask was evaporated, and the residue, consisting of pure picric acid (mp 121.5°–122° C), was weighed.

Picric acid from UCD Calc: 67.26%
Picric acid from murexine picrate
Found: 67.70%–67.10%–67.30%

In the nitron method, the determinations, also gravimetric in this case, were performed on aliquots of 0.2 g of murexine picrate, dissolved in 60 ml of hot water.

Nitron picrate from UCD Calc: 158.6%
Nitron picrate from murexine picrate
Found: 158.4%–157.8%–160.4%

Murexine hydrolysis products. The positivity of the Feigl hydroxylamine reaction (*4*) confirms that murexine is an ester of a carboxylic acid. The substance saponifies quickly in alkaline medium, more slowly in acid medium (heating in boiling water bath for 1–2 hr with 1 *N* HCl), liberating choline (I) and the acid which esterifies choline (II).

For various reasons the acid hydrolysis was preferred. After the picric acid was quantitatively removed with ether and the acid aqueous liquid was evaporated to dryness, there remained a white deposit, rather hygroscopic, which was brought to constant weight over phosphorus pentoxide *in vacuo*.

From two aliquots of 1 g each of murexine picrate, 476 and 467 mg of material were obtained.

$C_5H_{14}ON \cdot Cl$ (I) + $C_6H_6O_2N_2 \cdot HCl$ (II) Calc: 46.11%
from UCD

[1] We are indebted to, and wish to thank, F. Canal, of the Farmitalia Research Laboratories, for the elementary analyses, and K. Bernhardt, of the Biochemical Department of the University of Basel, for a sample of synthetic urocanic acid.

Choline (I). The presence of choline among the hydrolysis products of murexine was ascertained chemically (preparation of a reineckate identical to that of choline), by paper chromatography (*5*), and pharmacologically (the acetylation of the murexine hydrolysis products gives a substance which cannot be distinguished from acetylcholine).

Choline was determined quantitatively by direct weighing, following its precipitation as Reinecke salt. Aliquots of 200 mg of murexine picrate were used in the determinations.

$C_5H_{14}ON \cdot C_4H_7N_6S_4Cr$	from UCD	Calc: 61.97%
	" murexine picrate	Found: 62.80%–62.85%
$C_5H_{14}ON \cdot C_4H_7N_6S_4Cr$		Calc: Cr 12.26%
Reineckate from murexine hydrolysis products		Found: 12.46%
" " choline chloride		" 12.44%

Choline esterifying acid (II). Having demonstrated that murexine picrate contained 2 molecules of picric acid and 1 of choline, we were compelled, on the basis of the elementary analyses, to acknowledge the presence of 2 nitrogen atoms in II. Owing to the positivity of the Feigl reaction, also, the presence of 2 oxygen atoms became extremely probable.

A decisive step forward in the identification of product II was made by observing that it gave, in alkaline medium, an intense coupling reaction with diazonium salts, above all with the Pauly reagent. The color reactions of phenols, indoles, purines, and pyrimidines were, on the contrary, negative (these substances were also excluded through the ultraviolet spectrophotometric analysis). On the basis of these findings our orientation toward an imidazole derivation of the substance became more and more justified.

Chromatography on paper enabled us to discard the identification of product II with β-[imidazolyl-(4)]-propionic acid, and definitely to establish its identity with β-[imidazolyl-(4)]-acrylic acid, a possible intermediate product of the histidine metabolism.

A complete and easy separation of the choline chloride from the hydrochloride of the β-[imidazolyl-(4)]-acrylic acid was brought about by chromatographing the murexine hydrolysis products on a column of Whatman cellulose powder. *n*-Butanol saturated with 1 *N* HCl was used as solvent.

The hydrochloride of the β-[imidazolyl-(4)]-acrylic acid was finally transformed into the β-[imidazolyl-(4)]-acrylic acid, which is much less soluble in water and therefore more easily crystallizable and purifiable.

Needles, mp 217°–218° C, mixed mp with synthetic urocanic acid 217°–218° C.

$C_6H_6O_2N_2 \cdot 2H_2O$ (174)	Calc:	41.17	H 5.88	N 16.47%
Hydrolysis product II	Found:	41.25	5.93	16.41

β-[Imidazolyl-(4)]-acryl-choline dipictrate has been recently obtained by synthesis in our laboratories (*6*). It is identical with murexine picrate, both from the chemical and the pharmacological point of view.

Extensive pharmacological researches are now in progress on murexine and similar synthetic substances.

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The Effect of Estrogen on the Isometric Tension of Rabbit Uterine Strips

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It has been shown in our laboratory (*1*) that the concentration of uterine actomyosin, the contractile protein of the myometrium, is under the control of the ovarian estrogenic hormones. When rabbits in natural estrus were subjected to bilateral ovariectomy the concentration of uterine actomyosin decreased to about one fifth the original estrous value. This extreme condition could be reversed, however, by the administration of estrogen to the living animal, which brought about gradual increase in actomyosin concentration toward the estrous value.

Previous observations (*2, 3*) also indicated that similar changes in the concentration of uterine actomyosin occur under physiological conditions (pregnancy, labor, and menopause) that involve alterations in the estrogen output of the animal's own ovaries.

It has been generally accepted and has become a textbook statement that the fibrous protein actomyosin is the final contractile substance of muscle. The direct evidence in support of this conclusion—namely, Szent-Györgyi's actomyosin-thread "contraction" phenomenon—has not been accepted by some authors (*5, 6*). Evidence, however, may be obtained indirectly by utilizing the above-mentioned experimental variability of actomyosin concentration in uterine muscle. One would expect that the concentration of the final contractile substance would determine the maximum force the muscle is able to develop, provided that conditions are otherwise optimal. The existence of such a relation between actomyosin concentration and maximum tension will be demonstrated if the former is varied in a given muscle and proportionate alterations in maximum tension are then observed.