

It is possible that a combination of superfetation late in pregnancy, combined with delayed implantation of the blastocysts, may account for the observed results. However, the case of the *dba* mouse is difficult to explain on this basis, inasmuch as 10–15 days must have intervened between the last possible mating and the implantation of the blastocysts. Parthenogenesis is improbable because at least two of the last litters were made up of male and female animals.

This phenomenon may be more common than the literature would indicate. Two people who breed animals for experimental work have told the author that they have observed it occasionally, but did not have adequate records on individual animals. The only reference the author has been able to find of such cases is the one by Burrows (5), who reports ten examples in Wistar rats and two in *dba* mice. Five of these animals had records of the date of isolation, and in these cases the intervals between isolation and the birth of the second litter were 24–29 days. Burrows quotes two references to this phenomenon which he found in the literature, involving two cases in mice and one case in a rabbit. This anomalous type of pregnancy is not confined to any one genus, having been observed in the cases reported here, in *Rattus*, *Mus*, and *Peromyscus* (6). It is not confined to highly inbred strains, as the *Peromyscus* mouse was one of a stock carrying both white coat color and hairlessness, apparently as recessive characteristics.

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## The *in Vivo* Conversion of Glycine to Serine

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The conversion of glycine to serine by the liver has been shown to occur *in vitro*. A similar reaction has not been previously demonstrated in the liver of the intact animal. It will be shown that under varying conditions the metabolic path of glycine in the liver of the intact mouse is similar to the *in vitro* reaction.

Nine "A" strain male mice were equally divided into three groups. Group I was supplied with its normal diet, Group II was given only a saturated solution of glucose in water, and Group III was allowed only water. This diet was continued for 72 hr.

Each mouse was then injected with 1.728 mg of

glycine-2-C<sup>14</sup> (8.6  $\mu$ C) of C<sup>14</sup>-methyl-labeled glycine, and one mouse from each group killed with ether 2, 4, and 6 hr after the administration of the radioglycine.

TABLE 1

Group	Time (hr)	Amino acids	Relative radioactivity
I (Normal diet)	2	Glycine	++++
	4	"	++++
	6	Serine	++++
		Glycine	++++
II (Glucose + water)	2	Glycine	++++
		Serine	++
	4	Glycine	++++
		Serine	+++
	6	Glycine	++
		Serine	++
III (Water alone)	2	Glycine	++++
	4	"	+++
	6	Cystine	+
		Glycine	+++
		Cystine	+
		Serine	+++

The liver was removed *in toto* from each animal and hydrolyzed in 6 N HCl over a steam bath for 18 hr. The hydrolysate was filtered, and salts were removed by ion exchange technique. The solution was concentrated and chromatographed two-dimensionally on No. 1 Whatman filter paper (1, 2), using phenol and butanol propionic acid. After the papers had dried, they were radioautographed on Eastman No-Screen x-ray films. The amino acids on the chromatograms were then developed with a spray of 0.1% alcoholic ninhydrin. By superimposing the respective radioautographs on the developed chromatograms the radioactive amino acids could be determined. This activity must have been derived from the injected radioglycine. A small fragment of each liver was also examined microscopically to determine any cytological changes that were due to diet.

Table 1 summarizes the results. Two hr after injection, only the glucose-fed animal produced a moderate amount of radioactive serine. After 4 hr the glucose-fed animal had produced a little more radio-serine, and in the normal-fed animal radioglycine and radioserine were present in approximately equal concentrations. Only after 6 hr did the water-fed animals convert glycine to serine. In addition they showed the presence of a small amount of radiocystine not present in either of the two other groups.

Liver biopsy revealed no abnormality in the normal group, minimal vacuolization was present in the glucose-fed animals, and marked degenerative changes were seen in the water-fed group.

Winnick et al. (3) showed that after incubation of liver homogenate with C<sup>14</sup>-labeled glycine, 60% of the isotopic carbon was found in serine. Sakami (4) felt that such conversion occurred by means of the combination of glycine with formate and provided

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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  $1\frac{1}{2}$  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

### Identification of Murexine as $\beta$ -[Imidazolyl-(4)]-Acryl-Choline

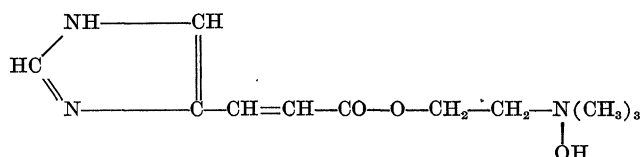
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Murexine is an active product contained, often in very large quantities, in the median zone of the hypobranchial body of *Murex trunculus* and of other related species of mollusks. Biologically, it manifests intense nicotinic and curariform actions; 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  $\beta$ -[imidazolyl-(4)]-acryl-choline or 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<sub>11</sub>H<sub>18</sub>O<sub>2</sub>N<sub>3</sub> · C<sub>6</sub>H<sub>3</sub>O<sub>7</sub>N<sub>3</sub> · C<sub>6</sub>H<sub>2</sub>O<sub>7</sub>N<sub>3</sub> (681)  
Murexine picrate (Sample 1)  
“ “ (Sample 2)  
“ “ (Sample 3)

*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

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there is the suggestion of an additional metabolic path in the production of radiocystine.

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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 mu-

*Murexine*  
(Urocanyl-choline)

rexine 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

Calc:	C 40.53;	H 3.35;	N 18.50%
Found:	40.68	3.57	18.34%
"	40.55	3.60	18.71%
"	40.51	3.60	18.70%

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.

$$\text{C}_5\text{H}_{14}\text{ON} \cdot \text{Cl} \text{ (I)} + \text{C}_6\text{H}_6\text{O}_2\text{N}_2 \cdot \text{HCl} \text{ (II)} \quad \text{Calc: 46.11\%}$$