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The distribution of atropinesterase and homatropinesterase in rabbit and guinea-pig liver cell fractions and sera

Ever since SCHROFF[1] first noted in 1852 that some rabbits could eat belladonna leaves with no apparent ill effects, there has been an interest in the metabolism of atropine and related alkaloids in various species. One of the metabolic processes utilized for atropine detoxication in some species is the enzymic hydrolysis of the ester linkage. However, BERNHEIM[2] has stated that hydrolysis of atropine is probably not an important detoxication mechanism. In addition to BERNHEIM, papers by AMMON AND GAVELSBURG[3] in 1949 and CIHAK[4] in 1960 contain good reviews of the research on atropinesterases which had been carried out up to the time of publication of the respective papers. Information concerning this subject is controversial. Many investigators have stated that an esterase is present in the sera and the liver of approximately two-thirds of the rabbit population and that it can be demonstrated *in vitro*[5–10,12,13]. However, as recently as 1949, GODEAUX AND TONNESON[13], using *in vitro* methods claimed that rabbit liver contained no hydrolytic activity, a finding they shared with DESARAM[21]. Because the esterase in rabbits is genetically controlled[3,11], it is possible that these authors were working with rabbits devoid of esterase. Again, other investigators[8,9,12,20] have stated that guinea-pig liver contains an esterase which will split atropine whereas others[14] have given evidence to show that although homatropine is hydrolyzed by guinea-pig liver, atropine is not.

The more recent work of WERNER[12] and MARGOLIS AND FEIGELSON[16] indicated the following: approximately two-thirds of the rabbit population contain an esterase (tropinesterase) which hydrolyzes (−)-hyoscyamine 80 times faster than (+)-hyoscyamine. The former is the naturally occurring component of belladonna which on completion of the extraction procedures is racemized to atropine which is a mixture of the two optically active isomers. The enzyme is relatively non-specific. Mixtures of atropine and other substrates result in mutual inhibition[16].

The results obtained from studies performed in our laboratory demonstrated that guinea-pig liver did not hydrolyze atropine. This is contrary to the results obtained by WERNER[12] and others[8], and it was decided to study in greater detail the site of action of the enzymes in rabbit and guinea-pig liver cells with the hope of gaining knowledge as to whether one or two enzymes are responsible for the hydrolysis of the two different substrates.

Male and female New Zealand White rabbits purchased from various commercial sources were used in this study. Rabbits of the (−)-hyoscyamine-positive types were chosen by screening for the esterase in blood samples taken from the ears. The guinea pigs were mainly Hartley strain from a colony maintained at Suffield since 1954. However, guinea pigs for two tests were obtained from two outside sources; a commercial source in Montreal, Quebec, and a laboratory in Lethbridge, Alberta, Canada.

The animals were killed by a blow on the neck. The thoracic cavity was then opened up and the liver was perfused by retrograde circulation of cold saline through the inferior vena cava.

Two methods of fractionation were used (a) the method of ADIE AND TUBA[17]

and (b) the method of SPORN[18]. The latter method was used in some rabbits in an attempt to improve the quality of the nuclear fraction obtained. However, as very little difference was noted in the specific activity of the nuclear fraction, the results were combined.

The nitrogen content of the fractions was determined using a micro-Kjeldahl technique with steam distillation followed by titration.

Esterase determinations were carried out initially employing a Warburg apparatus using the method outlined by GLICK[10] and later a Radiometer titrator recorder set up to record the volume of base required to maintain a constant pH. The titration was carried out using either 0.02 M or 0.04 M NaOH. The reaction chamber was kept at a constant temperature of 38°. 1 ml of diluted tissue and 1 ml of water were pipetted into the chamber and the mixture was allowed to equilibrate at pH 7.80. 100 μl of a 20% solution of either atropine or homatropine were added and the quantity of base needed to maintain a constant pH of 7.80 was recorded. The activity was calculated in terms of μM/min per mg N (ref. 19). The reaction rate was constant over a 2-h recording period.

Table I shows the results obtained with ten rabbits and eleven guinea pigs.

TABLE I

COMPARISON OF ATROPINE AND HOMATROPINE HYDROLYSIS BY RABBIT AND GUINEA-PIG LIVER FRACTIONS AND SERUM

Liver cell fractions	*Specific activity (μM/min per mg N)*				
	Rabbit liver		*Ratio A/B*	*Guinea-pig liver*	
	Atropine (A)	*Homatropine (B)*		*Atropine*	*Homatropine*
Nuclei	35.8 ± 4.3	24.5 ± 3.6	1.4	nil	32.3 ± 4.3
Mitochondria	26.1 ± 3.7	15.9 ± 3.0	1.6	nil	54.2 ± 4.6
Microsomes	30.3 ± 4.6	19.5 ± 4.8	1.6	nil	66.4 ± 7.7
Supernatant	nil	nil	—	nil	nil
Serum	16.3 ± 2.7	9.2 ± 1.8	1.8	nil	nil

In rabbits the order of activity in the cellular fractions for both substrates was nuclei>microsomes>mitochondria>serum. The ratio of atropine to homatropine hydrolysis was essentially constant for each fraction. In guinea pigs the order of activity for homatropine was microsomes>mitochondria>nuclei with serum having no activity. There was no activity in the supernatant fractions for either species. None of the guinea-pig liver fractions tested hydrolyzed atropine. In view of this, further tests were made on guinea pigs obtained from two other sources, one in Montreal, Quebec, and one in Lethbridge, Alberta. A total of 24 such animals were tested for the ability of liver homogenate to hydrolyze atropine. None of the animals possessed atropinesterase activity.

In view of the variation in distribution, the difference in substrate selectivity and the constant ratio of atropine to homatropine hydrolysis in rabbit-liver fractions one can only assume, as did WERNER AND BREHMER[12] with other evidence, that there is a difference between tropinesterase, rabbit atropinesterase and guinea-pig liver homatropinesterase which, by some investigators[8,20], has been confused with atropin-

esterase. Unlike WERNER AND BREHMER[12], but in agreement with GLICK AND GLAUBACH[14] and ELLIS[15], we were not able to demonstrate any activity in guinea-pig liver or serum towards atropine. MARGOLIS AND FEIGELSON[16] have suggested that rabbit atropinesterase is non-specific on the basis of competitive inhibition studies. Our evidence also indicates that a single non-specific enzyme is responsible for the hydrolysis of both atropine and homatropine in the rabbit. It also shows that a different more specific enzyme is responsible for the hydrolysis of homatropine in the guinea pig.

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Oscillationen der Ureaseaktivität von Hydrogenomonas H 16 in statischer Kultur

Die Urease von Hydrogenomonas H 16 ist als katabolisches Enzym anzusehen und wird anscheinend durch katabolische Repression (durch Ammonium-Ionen) und eine bei N-Mangel eintretende endogene Derepression[1] reguliert. Die durch Harnstoff "induzierte" Enzymbildung setzt Bedingungen voraus, unter denen auch eine endogene Derepression erfolgt. Während des Wachstums in statischer Kultur mit Ammoniak als N-Quelle enthalten die Zellen von Hydrogenomonas H 16 nur wenig Urease; die spezifische Ureaseaktivität beträgt im Mittel 7 Einheiten/g Protein. Die