The Nature of Rabbit Serum Cholinesterase.* (17590)

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The cholinesterases have been studied extensively in recent years especially in relation to nerve activity. However, the demonstration that more than one enzyme can split acetylcholine has disclosed the existence of a non-specific enzyme which plays no known role in nerve activity.(1) This enzyme is present in the serum and tissues of many species including man. Most of the research on it has been directed either toward the identification of this enzyme and its separation from the specific form, or toward its quantitative variation in disease states. In a previous paper,(2) we reviewed the recent literature on the variation of this enzyme in various pathological states, and concluded that in addition to our finding of a decrease of the non-specific enzyme in serum in pregnancy and patients with malignancies, there was satisfactory evidence in the literature of a decrease in the level of this enzyme, in pernicious anemia in relapse, and in liver damage. Further study in our laboratory of this phenomenon revealed that the human serum cholinesterase (the non-specific enzyme) did not vary in specific diseases, but rather varied directly with the serum albumin in all diseases except those with albuminuria. The results of this work are published elsewhere.(3)

The fact that the serum cholinesterase decreases in liver damage suggests the possibility that the liver is involved in the synthesis of both the albumin and the cholinesterase, but the exact relationship of one to the other remains to be discovered. With this in mind we went to an experimental animal, the rabbit, to work out the theoretical possibilities. The rabbit has been reported by Mendel and Rudney(4) to have both the specific and non-specific enzyme in its serum, but Ellis and co-workers(5) were unable to produce a decrease in the serum cholinesterase in this animal after liver damage. Their work was inconclusive, however, since no albumin determinations were carried out. Because of this, we decided to study the cholinesterase enzyme pattern in the rabbit before using it for our principal experiments.

Method. In the tests for serum cholinesterase, we have followed the procedure of Mazur and Bodansky,(6) who modified the method of Ammon.(7) The production of acid from the substrates was followed at 37°C by means of the liberation of carbon dioxide from a bicarbonate-carbonic acid buffer in Warburg flasks. These vessels were gassed at room temperature with a mixture of 93% oxygen and 7% carbon dioxide. At zero time, the substrate was tipped into the serum and bicarbonate mixture. Readings were made at 20 minutes at which time the readings fell on a zero order curve. 0.25 ml of rabbit serum was used in a final volume of 3.00 ml, .04 molar in sodium bicarbonate. Final substrate concentrations were: acetylcholine 0.015 M, benzoylcholine 0.006 M, and acetyl-β-methylcholine 0.03 M.

Results. The assumption that the rabbit has both the specific and non-specific cholinesterases is based on the hydrolysis of the two substrates acetyl-β-methylcholine (hydrolyzed by the specific form), and benzoylcholine (hydrolyzed by the non-specific type), in addition to acetylcholine (hydrolyzed by both). In our own experiments, the examination of the ratios of the 3 substrates revealed a fairly constant ratio of acetyl-β-methylcholine to

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TABLE I.
Ratios of the Hydrolysis Rates of Benzoylcholine and Acetyl-β-methylcholine by Equal Amounts of Rabbit Serum.

Rabbit	ACh	BCh	MCh	BCh/ACh	MCh/ACh
66	28.7	9.5	13.3	.33	.47
69	39.1	34.3	16.5	.96	.42
70	52.8	32.7	21.7	.62	.41
52	39.4	6.7	15.4	.17	.39
59	35.7	20.9	11.2	.59	.32
60	34.7	18.6	11.7	.54	.34
64	32.4	4.8	11.1	.15	.35
122	48.6	71.3	15.7	1.5	.32
Aero.	62.0	39.4	27.6	.64	.45
57	62.7	81.4	19.0	1.3	.30
55	36.5	30.1	12.5	.82	.34
54	43.1	55.2	18.2	1.3	.42
53	39.4	29.3	19.3	.74	.49
Gaud.	43.6	47.9	19.8	1.1	.46
81	53.5	27.1	19.2	.51	.36
83	39.4	33.2	11.7	.84	.30
84	48.6	39.1	18.8	.81	.39
85	62.8	30.6	24.4	.49	.39
87	49.0	33.2	14.8	.67	.30
88	52.6	44.5	21.5	.85	.41
89	56.9	43.2	23.6	.76	.42
90	66.5	41.1	23.1	.62	.35
91	62.0	112.0	23.8	1.8	.30
82	48.2	42.9	21.0	.89	.44
93	47.2	5.2	22.8	.11	.49
94	48.7	35.0	17.6	.72	.37

TABLE II.
Effect of Pontocaine upon Cholinesterase Activity.*

Source of enzyme	Inhibition of hydrolysis of		
	Acetylcholine %	Benzoylcholine %	Acetyl-B-methylcholine %
Human Serum	100	2	—
Hemolyzed Human Erythrocytes	0	—	0
Rabbit Serum	2	7	11
Hemolyzed Rabbit Erythrocytes	4	—	12

*Final concentration of pontocaine hydrochloride 10^{-4} M.

acetylcholine hydrolysis, regardless of whether the rabbit serum contained an enzyme hydrolyzing benzoylcholine or not. On the other hand, there was no constant relationship between the benzoylcholine and acetylcholine hydrolysis (Table I). This would indicate that the enzyme which hydrolyzes benzoylcholine in the rabbit either does not hydrolyze acetylcholine or at best hydrolyzes it in insignificant quantities. This is unlike the human non-specific serum cholinesterase, which gives a constant ratio of the hydrolysis of benzoylcholine to acetylcholine. The average ratio of benzoylcholine to acetylcholine hydrolysis, for equal amounts of serum, for 42 normal individuals, as determined in this laboratory, was 0.345 with a standard deviation of 0.04. Thus we must conclude that the enzymes hydrolyzing benzoylcholine in the rabbit and the human are different and that measurement of acetylcholine hydrolysis alone would yield no information in the rabbit as to the enzyme hydrolyzing benzoylcholine.

Further evidence that the enzymes hydrolyzing acetylcholine in the human and in the rabbit are different, and that very likely only the enzyme hydrolyzing acetyl-β-methylcholin attacks acetylcholine in the rabbit is presented in Table II.

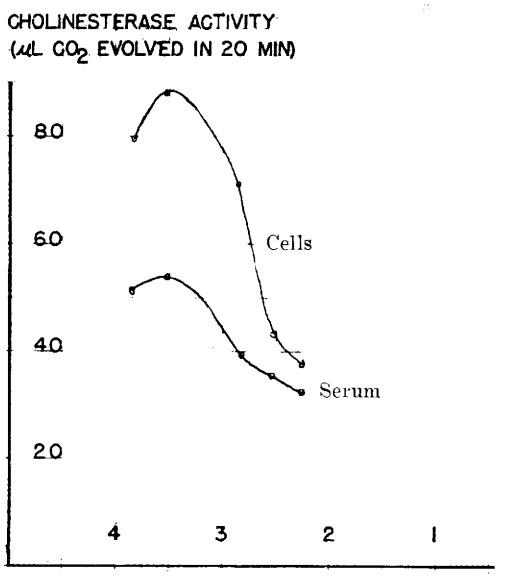

FIG. 1.
Activity-pS curves for rabbit serum and erythrocytes.

Ferrarai(8) has shown that pontocaine inactivates the non-specific cholinesterase but not the specific cholinesterase. Since the results in Table II indicate no inhibition by pontocaine of acetylcholine hydrolysis by rabbit serum, we must conclude that the enzyme hydrolyzing acetylcholine in the rabbit is not the same one hydrolyzing it in human serum.

Evidence that the serum enzyme in the rabbit is the true type of cholinesterase is seen in Fig. 1. The activity-pS curve here is the bell-shaped curve characteristic for the specific or true cholinesterase and acetylcholine.(9) This may further be seen from the curve for the rabbit erythrocytes also presented, the erythrocytes of most mammalian species having the true cholinesterase.

Conclusions. Rabbit serum, contrary to previous observations, contains only one enzyme which hydrolyzes acetylcholine, and this one has the characteristics of a true cholinesterase. In addition, there is present in rabbit serum an enzyme which hydrolyzes only benzoylcholine, and which, since it does not hydrolyze acetylcholine significantly, should not be labeled a cholinesterase. This enzyme may be similar to the "benzoylcholinesterase" described by Sawyer(10) as being present in guinea pig liver and kidney.

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Renal Hyperemia Induced in Man by a New Phthalazine Derivative. (17591)

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Pyrogen is the only substance which has been reported to increase significantly the renal blood flow in man. However, according to Goldring and Chasis,(1) it is not suitable for therapeutic purposes. Aminophylline seems to be effective only in patients with cardiac failure.(2) No treatment which will relieve renal ischemia has yet been found.

According to Meier *et al.*,(3) Compound 5968 (hydrazino-phthalazine hydrochloride), supplied by Ciba, produces in animals a sustained fall in blood pressure. In man, we found that hypotension did not occur regularly following the subcutaneous injection of 10 mg. However, when measurements of renal plasma flow were performed, using the

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