

at a rate of 10 per minute. The magnitude of the exchange afforded by fetal respiratory movements was illustrated by lungs removed within the first minute following introduction of ink within the amniotic sac. Darkening of the lung was clearly evident.

Observations are based upon 25 fetuses obtained from 7 rabbits. Fourteen fetuses were breathing at the time of injection while 11 were apneic.

In conclusion, it is clear that amniotic fluid normally occupies the alveoli of the fetal lung. The spontaneous respiratory movements of the fetus are responsible for a tidal flow in the respiratory tract. There is evidence that intrauterine respiration is of functional significance in the development of a normal lung, aiding in dilatation of the alveoli and elastic walls of the future air passages.

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Choline-Esterase Activity of Human Sera, with Special Reference to Hyperthyroidism.*

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(Introduced by Harry Sobotka.)

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Until 1932, the rate of enzymatic destruction of acetylcholine was determined biologically. In that year, Stedman¹ and his co-workers described a chemical method for assaying the acetylcholine splitting enzyme in the blood by titrating the acid liberated from the hydrolysis of acetylcholine at a constant pH. This esterase also acted upon butyrylcholine. In 1933, they found that the choline-esterase of human serum was relatively high and showed considerable individual variation.² This activity also varied widely in the different species.

In 1933, Ammon³ employed a gasometric method by which he determined the amount of carbon dioxide liberated from a carbonate solution by the acid formed from the hydrolysis of the acetylcholine substrate. The carbon dioxide was measured in the Bar-

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

¹ Stedman, E., Stedman, E., and Easson, L. H., *Bioch. J.*, 1932, **26**, 2056.

² Stedman, E., Stedman, E., and White, A. C., *Bioch. J.*, 1933, **27**, 1055.

³ Ammon, R., *Pflügers Arch. f. d. ges. Physiol.*, 1933, **233**, 486.

croft-Warburg respirometer, and the amount formed served as an index of the acetylcholine splitting power. In the meantime, Stedman independently devised a method, utilizing the Barcroft differential apparatus, which was based upon the same principle. The advantages of these methods far surpassed the titration procedure since red blood cells and colored solutions could be tested, whereas the pigments interfered with the older procedure.

In 1935, Ammon and Voss⁴ showed that human sera had a weaker splitting activity than whole blood. They also confirmed Stedman's 1932 findings of marked variations of human blood, and in addition, found that individuals had constant splitting powers over a period of several weeks.

In 1936,⁵ Von Verebely, returning to a biological method, confirmed Ammon's findings.

We have made upwards of 500 determinations on unselected hospitalized adults and 60 selected, apparently normal individuals of essentially the same age group as the pathological cases. We used Ammon's procedure,³ except that the reaction was permitted to continue over a 2-hour period in the water bath at 30°C. and that the spontaneous hydrolysis of acetylcholine was determined with each experiment and this result subtracted from the total figure.† The first hundred determinations were performed in duplicate and the differences between the duplicates were found to be insignificant. In many of these, eserine in high dilutions (1:10⁸) completely inhibited the enzymatic scission. The control group consisted of professional blood donors and members of the hospital personnel. Sixty consecutive subjects in this so-called normal group revealed an average reading of 67.6 mm³, with all cases except 7 falling between 44 and 80 mm³. The exceptions were 31, 81, 82, 83, 86, 95, and 99. (Table I.) It was not possible to determine the causes for these extreme figures since many of this group were not available for further study. Many of the controls were repeated over a period of weeks and the activity of the sera obtained from the same individual at different times remained virtually constant. A number of sera in this group obtained in the fasting state and after meals revealed but slight variation. Almost all of the specimens in this series, however, were obtained in the fasting state. In one case, blood was taken at 4-hour intervals for a period of 24

⁴ Ammon, R., and Voss, G., *Pflügers Arch. f. d. ges. Physiol.*, 1935, **235**, 393.

⁵ Von Verebely, T., Jr., *Kl. Wchnschr.*, 1936, **15**, 11.

† We have found that the spontaneous hydrolysis of the acetylcholine solutions, which were made up separately for each experiment, varied despite the fact that the salt was obtained from the same bottle.

CHOLINE-ESTERASE OF HUMAN SERA

TABLE I.
Control Group.

31	44	50	60	70	80	95
	47	52	60	71	81	99
	47	53	60	71	82	
		53	61	71	83	
		55	62	71	86	
		56	63	71		
		57	63	71		
			63	72		
			64	73		
			64	73		
			64	74		
			64	74		
			65	75		
			65	75		
			65	75		
			67	75		
			67	76		
			69	76		
			69	77		
			69	78		
				78		
				79		

Choline esterase activity of serum expressed in mm³. CO₂ developed in 2 hours at 30° C.

TABLE II.
Cases of Hyperthyroidism.

Untreated (22 Cases)	Treated (13 Cases)
117	100
109	85
109	85
104	83
103	77
102	74
99	73
98	70
97	68
96	66
90	62
89	58
87	49
87	
87	
81	
81	
80	
76	
71	
62	
62	
Average 90 mm ³ .	73 mm ³ .

Choline esterase activity of serum expressed in mm³. CO₂ developed in 2 hours at 30° C.

hours and the figures varied from 54 to 62 mm³., the highest figure being obtained at 7:00 A. M. and 10:00 A. M., and the lowest at

10:00 P. M. We could not obtain blood during sleep because a subject was not available into whose vein we could tie a cannula.

In the hospitalized cases we found the variations in the hydrolyzing effect to be much more pronounced. The figures varied from 8 mm³. of carbon dioxide to 163 mm³. of carbon dioxide liberated in a period of 2 hours. The average of these readings was 62 mm³. All of these specimens were obtained in the fasting state. The disease groups fell within broad but definite ranges. Thus, the group consisting of 35 unselected clinical cases of hyperthyroidism averaged 84 mm³. Of these, in 22 non-treated cases, the average was 90 mm³., while the other 13 which had been treated either by lugo-lyzation or operation, showed an average of 73 mm³. (Table II.) The treated case with 100 mm³. was an instance in which symptoms recurred 2 years after thyroidectomy. A case of nephrosis, not included in this series, which had been receiving thyroid therapy split 129 mm³. No untreated case of nephrosis was available for comparison.

The recognition of the muscarine action of acetylcholine by Dale, the demonstration by Loewi of a substance formed as a result of vagus stimulation, the so-called "Vagusstoff", which is responsible for transmission of the parasympathetic impulse and which is a choline ester closely resembling acetylcholine, and in its effects, virtually identical with acetylcholine, the evidence of destruction of this substance by an esterase which is physiologically indigenous throughout the body, in conjunction with the findings herein recorded, lend support to the belief that the choline esterase activity may be one of the elements related to the so-called vagotonicity or sympathicotonicity of an individual. Inferentially, this choline-esterase activity may be a factor which is inversely related to the vagotonicity and parallels the sympathicotonicity of the individual. The fundamental basis for this hypothesis centers about the individual variations in the potency or amount of choline-esterase, determining the rate of destruction of the vagus substance. In the so-called sympathicotonic person there may be an increased amount of or more potent enzyme; in vagotonic individuals, there may be less enzyme or a weaker enzyme. The former type would obviously destroy the vagus substance rapidly, influencing the ensuing autonomic equilibrium in such a manner that the sympathetic factors are comparatively increased. The latter type may destroy it more tardily, thus sparing the vagus substance which remains relatively unaltered and capable of activity over a longer period of time with a result simulating a summation effect in which the parasympathetic factors predominate.

Although we have not sufficient data on diabetes mellitus, it appears that the non-treated severe diabetic figures approximate or surpass the upper limit of normal, dovetailing with the thyroid group, whereas the treated cases are grouped in the lower levels of the normal range. Some of the hypertensive cases also exceed the higher normal figures. On the other hand, many of the sera obtained from patients with jaundice, cirrhosis of the liver, hepatitis, anemia, arthritis, rheumatic fever, and hyperpyrexia reveal pronouncedly depressed values. Further studies are now being conducted to clarify the significance of these results.

Summary. The acetylcholine esterase activity of the blood serum was determined in 500 individuals including normal and pathological cases. A modification of the Ammon gasometric method was utilized. The acetylcholine esterase was relatively high in cases of untreated hyperthyroidism.

The acetylcholine esterase activity may be one of the elements related to the so-called sympathicotonicity or vagotonicity of an individual.

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Hepatic Excretion in Man of the Various Bile Acids Following Their Oral Administrations.

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It is well known that animals normally excrete in their bile the various bile acids specific to their species. When foreign bile acids are administered by mouth they are readily absorbed and excreted by the liver. Weiss¹ as well as Prevost and Binet² showed that orally administered, sodium glycocholate, ordinarily absent in dog's bile, is excreted in the bile. Jenke³ found an excess of free cholic acid in the bile of a dog after feeding that acid in large amounts.

Human bile contains principally a mixture of cholic and desoxycholic acid in approximately equal proportions; about four-fifths of

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¹ Weiss, A., *Centralbl. f. die Med. Wiss.*, 1885, **23**, 121.

² Prevost, J. L., and Binet, Paul, *Comp. rend. acad. de. sc.*, Paris, 1888, **106**, 1690.

³ Jenke, M., *Arch. f. exp. Path. u. Pharmakol.*, 1928, **130**, 280.