These phasic changes are regarded as being implemented chiefly, but not necessarily solely, by the accumulation of hydrogen peroxide, which may be accounted for on the basis of our demonstration of the anticatalase-action of the sulfonamide compounds.[3-6]

11014

Choline Esterase Activity in Blood Serum and Duodenum of Beriberi Pigeons.

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It has been recently demonstrated by Glick and Antopol[1] and by Süllmann and Birkhäuser[2] that thiamine exerts an inhibitory action upon choline esterase *in vitro*. Zeller, Schär and Staehlin[3] showed that the splitting of histamine and diamines by the diamino-oxidase is also inhibited by thiamine. It has been shown by Goodhart and Sinclair[4] that cocarboxylase is contained in the cellular elements of the blood and the free thiamine essentially in the plasma. In contrast to thiamine, cocarboxylase recently has been found to have very little inhibitory effect upon the enzymes.[5] The question then arises whether thiamine has a physiological effect, *in vivo*, on choline esterase. To throw light on this point measurements were made of the activity of the enzyme in the serum of beriberi and normal pigeons. The concentration of the enzyme in the small intestine was also measured and correlated with its sensitivity to acetylcholine.

Twenty-two pigeons in 3 series were used in these investigations. In the first group, 6 pigeons were placed on a polished rice diet and choline esterase determinations were made about every 8 days on blood serum obtained from the wing vein. When the pigeon showed

3 Locke, A., Main, E., and Mellon, R. R., *Science*, 1938, **88**, 620.

4 Main, E., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

5 Shinn, L. E., Main, E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 591.

6 Shinn, L. E., Main, E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 640.

1 Glick, D., and Antopol, W., *J. Pharm. Exp. Therap.*, 1939, **65**, 389.

2 Süllmann, H., and Birkhäuser, H., *Schweiz. Med. Wchnschr.*, 1939, **69**, 688.

3 Zeller, E. A., Schär, B., and Staehlin, S., *Helv. Chim. Acta*, 1939, **22**, 837.

4 Goodhart, R. S., and Sinclair, H. M., *Biochem. J.*, 1939, **33**, 1099.

5 Glick, D., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 396.

nervous symptoms, the estimations were made at suitable shorter intervals.

In the second and third group, consisting of 6 and 10 pigeons each, the birds were first maintained on a diet consisting of oats, corn, and water for a period of 2 weeks in group 2, and 3 months in group 3. During this time repeated choline esterase determinations were made on the sera After an initial period of acclimatization, the enzyme concentration remained constant. Each group was then subdivided into 2 series. One of these was continued on the full diet while the other was transferred to one of polished rice. When polyneuritic symptoms were observed, measurements of choline esterase were again carried out. Parallel determinations were made on the normal group. At the height of the beriberi stage, the choline esterase determinations were made once again and the birds sacrificed by decapitation.

The duodenum was immediately removed for a distance of about 2 cm on each side of its first angulation. The distal portion was severed from the proximal, opened, and washed free of its contents with Ringer solution. Great care was taken to remove the bile, since it has been shown to inhibit the enzyme.[6, 7] The duodenal choline esterase was determined in the same manner as previously employed for other tissues.[8]

The proximal loop of duodenum was washed through with cold oxygen-saturated Locke's solution, and stored for 4 hours in the ice chest immersed in this solution. The sensitivity to acetylcholine was then determined by the Magnus method.

The serum choline esterase was determined by the Ammon method at 30°C as previously described[7] employing 0.5 cc of solution prepared by diluting the serum 10 times with bicarbonate-Ringer.

The results for all 3 groups were essentially the same, hence only the data for group III are given (Table I). From March 14 to May 17 the 10 pigeons were on a full diet and showed fairly constant choline esterase levels in the blood. On May 15, the 5 pigeons were placed on a polished rice diet while the others remained on a normal diet. It is seen from Table I that, whereas the choline esterase of pigeons on a normal diet remain fairly constant, those on a polished rice diet show an increased choline esterase with the development of polyneuritic symptoms.

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6 Sobotka, H., and Antopol, W., *Enzymologia*, 1937, **4**, 189.

7 Antopol, W., Schifrin, A., and Tuchman, L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 363.

8 Glick, D., Lewin, A., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 28.

TABLE I.
Choline Esterase Activity of the Blood Serum of Normal and Beriberi Pigeons.

	Pigeon No.									
Date	15	16	17	18	19	20	21	22	23	24
2-19-39	All pigeons placed on normal diet									
3-14	144	170	138	135	120	146	129	165	128	190
3-16						163	152	170	144	186
3-26	162	158	137	134	122					
4-13	170	156	156	137						
4-17						117	111	160	125	183
5-12	158	154	159	141	147					
5-15						146	142	175	142	170
5-17	Remain on normal diet					Placed on polished rice diet				
6-12	167		166			200 (severe ataxia)		230 (severe ataxia)		
6-14		140		141	152				262 (severe ataxia)	240 (severe ataxia)
6-19						190* Opisthotonus				
6-20									225* (legs paralyzed)	200* (legs paralyzed)
6-22							223*			
6-23					155*					
6-27			130*							
6-28				129*						
6-29	137*									
6-30		140*								

* Killed.
Enzyme activity in blood serum expressed in mm³ CO_2 liberated in 1 hr at 30° C.

Similarly, there is an increased choline esterase concentration in the small intestine of beriberi pigeons, as well as a decreased sensitivity of the small intestine to acetylcholine (Table II). The decreased sensitivity had been already observed by Abderhalden and Abderhalden.[9] In contrast to these findings, Beauvallet[10] reported no change in the acetylcholine sensitivity of the small intestine of pigeons when the birds developed polyneuritis from a diet consisting of proteins, lipoids, glucose, and minerals. Both Abderhalden and Abderhalden, and Beauvallet found that the addition of vitamin B_1 to the insensitive small intestine of beriberi pigeons increased its reactivity to acetylcholine. Mintz[11] showed that vitamin B_1 increases the activity of acetylcholine on the isolated intestine of the rat. Since thiamine inhibits choline esterase, the question arises whether the diminished sensitivity of the small intestine of the

9 Abderhalden, E., and Abderhalden, R., *Pflug. Arch.*, 1938, **240**, 388.
10 Beauvallet, M., *Compt. rend. soc. biol.*, 1938, **128**, 1020.
11 Agid, R., Beauvallet, M., and Mintz, B., *Compt. rend. soc. biol.*, 1937, **126**, 982.

TABLE II.
Enzyme Activity of the Duodenum and Its Sensitivity to Acetylcholine in Normal and Beriberi Pigeons.

Date	Normal pigeons No.	Enzyme activity	Acetylcholine concentration
6-23-39	19	46	1: 5,000,000
6-27	17	56	1: 5,000,000
6-28	18	52	1: 8,000,000
6-29	15	64	1: 5,000,000
6-30	16	42	1:10,000,000
	Beriberi Pigeons		
6-19	20	68*	1: 100,000
6-20	24	140	1: 100,000
6-20	23	134	1: 50,000
6-22	22	132	1: 1,000,000

Enzyme activity expressed in mm³ CO_2 liberated in 1 hr at 30° C.
* Intestine not washed free of bile.

beriberi pigeon is due to the increased choline esterase, which, in turn, is dependent upon the absence of vitamin B_1 as has been previously suggested.[1] Our investigations confirm Abderhalden's findings of, first, a lack of spontaneous rhythmic intestinal contraction in beriberi pigeons, and, second, an increase in intestinal tone without production of rhythmic contractions after the addition of effective doses of acetylcholine. However, the small intestine of normal pigeons shows spontaneous rhythmic contractions and with acetylcholine there is first an increased tone and then an increase in intensity of the contractions. In addition, Abderhalden found that in the presence of thiamine, not only is tonus increased in the isolated intestine of beriberi pigeons, but rhythmic contractions occur. It appears, therefore, that vitamin B_1 is involved in the production of rhythmic contractions. The initiation of these rhythmic intestinal contractions may explain the findings of Frazier and Ravdin[12] that intestinal symptoms of hyperthyroid patients are alleviated by thiamine, since it was suggested that hyperthyroidism is associated with vitamin B_1 deficiency. This may be correlated with the observations of Antopol, Tuchman and Schifrin[13] that cases of untreated hyperthyroidism have relatively high choline esterase levels in the blood serum.

Summary. The choline esterase concentration was found to be increased in the serum and small intestine of beriberi pigeons. The small intestine of beriberi pigeons showed decreased sensitivity to

12 Frazier, W. D., and Ravdin, I. S., *Surgery*, 1938, **4**, 680.

13 Antopol, W., Tuchman, L., and Schifrin, A., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 46.

acetylcholine. The basis of these effects, and their significance has been discussed.

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11015

Prevention and Modification of Measles with Concentrated Pooled Ascites Fluid and with Its Globulin Fraction.*

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Various types of antibodies have been demonstrated in ascites fluid, but the presence of measles-protecting substances has not been reported. Since measles-protecting substances are found in the blood serum of most urban dwelling adults, it was thought of interest to test for their presence in a number of ascites fluids. Should these antibodies be present, the large amount of ascites fluid available in hospitals could provide an additional source of material for measles prophylaxis.

The ascites fluid was obtained from Wassermann negative individuals suffering from portal cirrhosis or cardio-vascular-renal disease. After Wassermann, Kline and sterility tests were found negative, a pool was made of aliquot parts of 9 fluids. Since less protein is present in ascites fluid than in serum it was realized that this fluid would have to be concentrated to provide a dose small enough to be practicable for intramuscular injection. The ascites fluid pool was divided into 3 parts. One portion was concentrated in sterile cellophane sausage casings by the corn syrup technic previously described,[1] another portion by the air technic,[2] and the globulin fraction was prepared by 50% ammonium sulphate precipitation.[3]

To obtain an amount of measles-protecting substances in concentrated ascites fluid equivalent to that in measles convalescent

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1 Thalhimer, W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 230.

2 Thalhimer, W., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 639.

3 Gibson, R. B., *J. Biol. Chem.*, 1906, **1**, 161.