After 28 days on the diet the limbs on right side of the body became spastic and extended. Later a right sided convulsive seizure occurred with head turned to right and right limbs rigid. One hour after intracisternal injection of thiamine all symptoms had disappeared. The diet was continued and 15 days later the right limbs were again spastic and frequent tonic convulsions involved the right side. Convulsions ceased after administration of 0.5 mg thiamine but a residue of spastic paralysis remained in the right hind limb. The animal died 17 days later whilst in thiamine deficiency for the third time.

The above findings may explain partial improvement with thiamine therapy of some neurological diseases which are not the direct result of vitamin deficiency.

Summary. 1. Subarachnoid injection of thiamine causes rapid recovery of thiamine-deficient cats. Recovery is equally rapid when the vitamin is given intravenously or intramuscularly. 2. Moderate signs of meningeal irritation follow introduction of thiamine chloride into the subarachnoid space. 3. Thiamine deficiency may unmask symptoms due to an organic defect in the brain.

13691

Effects of Cozymase upon Growth of Staphylococci and Antistaphylococcal Action of Sulfonamide Compounds.*

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Following the report of Knight[1] that thiamin chloride and nicotinic acid are essential growth factors for *S. aureus*, Gladstone[2] demonstrated that a medium of known chemical composition may be used for growth experiments with staphylococci. A chemically-defined medium has certain advantages for the study of the bacteriostatic effect of the sulfonamide compounds upon staphylococci.[3] West and Coburn[4] utilized Knight's "synthetic" medium for an investigation

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1 Knight, B. C. J. G., *Brit. J. Exp. Path.*, 1935, **16**, 315.

2 Gladstone, G. P., *Brit. J. Exp. Path.*, 1937, **18**, 322.

3 Spink, W. W., and Jermsta, J., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 395.

4 West, R., and Coburn, A. F., *J. Exp. Med.*, 1940, **72**, 91.

of the bacteriostatic effect of sulfapyridine upon the staphylococcus. They concluded that cozymase (Coenzyme I) interfered with the antistaphylococcal action of sulfapyridine, whereas nicotinic acid did not. Strauss, Dingle and Finland[5] were unable to confirm this finding. They made the interesting observation that cozymase could replace the essential growth factors, nicotinic acid and thiamin chloride, and suggested that impurities in the cozymase were responsible for this finding.

Because of the divergence of reported results concerning the effect of cozymase upon the bacteriostatic action of the sulfonamide compounds for the staphylococcus, we are recording our observations. An explanation is also presented for the confirmed observation that cozymase will stimulate growth of the staphylococcus in the presumed absence of nicotinic acid and thiamin chloride.

Material and Methods. The previously mentioned investigators used either a casein or gelatin hydrolysate as a source of amino acids. We employed a synthetic medium as described by Gladstone,[2] to which the individual amino acids were added. This precaution was taken in order to reduce the impurities to a minimum. A coagulase-positive strain of *S. aureus* isolated from a patient with osteomyelitis was used throughout. Two preparations of cozymase were investigated. One was supplied to us through the courtesy of Dr. D. F. Robertson of Merck and Company, and a second was prepared by one of us (O.M.) according to the method of Jandorf.[6] Professor C. A. Elvehjem of the University of Wisconsin found the activity of the second product to be 81% of the von Euler standard. Qualitative analysis showed that both preparations contained thiamin. The cozymase prepared by us was quantitated by the standard thiacrome method and found to contain 0.16 mg of thiamin chloride per mg of cozymase. As shown later, this amount of thiamin chloride was sufficient to support maximum growth of staphylococci when an optimum amount of nicotinic acid was present.

The basal medium contained all the essential ingredients except thiamin chloride and nicotinic acid. The culture for inoculation was first grown in the complete medium for 24 hours, then centrifuged and the bacterial cells washed with sterile saline before seeding the various tubes with a known number of organisms. The final volume in all the tubes was 10 cc. The degree of bacterial growth after incubation at 37°C was measured according to the turbidity of the contents in each tube. For this purpose, the Evelyn photoelectric

[5] Strauss, E., Dingle, J. H., and Finland, M., *J. Immunol.*, 1941, **42**, 331.

[6] Jandorf, B. J., *J. Biol. Chem.*, 1941, **138**, 305.

colorimeter was used as described previously.[3] Growth was expressed in terms of percentage of light transmitted through a standardized absorption tube. The uninoculated medium was usually water-clear and was utilized as the control standard.

Results. The first group of studies in which an experiment was designed to show the comparative growth stimulus of cozymase, and thiamin chloride and/or nicotinic acid and nicotinamide, in a basal medium containing small and large inoculae is summarized in Table I. A duplicate series of tubes was set up and the results recorded at the end of 24 and 72 hours of growth. From these and other similar observations, the following may be set forth. Thiamin chloride alone provokes only a slight growth response. The addition of only nicotinic acid to the basal medium results in a definite growth, particularly in the presence of a large inoculum. Nicotinamide will serve as a growth stimulus in a smaller concentration than will nicotinic acid, but the same degree of growth is attained when either compound is present in an optimum amount. Maximum, and essentially equivalent growth, was obtained when cozymase was added to the basal medium or when a combination of thiamin chloride and nicotinic acid or nicotinamide was added. A comparison of tubes 3, 4, 5, and 6 would indicate that thiamin chloride is at least one of the added factors responsible for maximum growth resulting with cozymase. A comparison of tubes 5 and 6 containing the small inoculum shows that preformed cozymase will initiate growth more rapidly than a combination of thiamin chloride and nicotinic acid, but that the degree of growth at the end of 72 hours is essentially the

TABLE I.
Comparative Effects of Thiamin Chloride and Nicotinic Acid (or Nicotinamide), and Cozymase on Growth of Large and Small Inoculum of *S. aureus.*

		A* Growth in % of light transmitted		B† Growth in % of light transmitted	
		24 hr	72 hr	24 hr	72 hr
1.	Basal medium	93	94	97	100
2.	" " + 0.0034 μg/cc thiamin chloride	94	88	97	93
3.	" " + 1.23 μg/cc nicotinic acid	75	79	96	88
4.	" " + 5.84 " " cozymase‡	39	41	52	41
5.	" " + 0.0034 μg/cc thiamin chl. + 1.23 μg/cc nicotinic acid	33	41	77	35
6.	" " + 0.00034 μg/cc thiamin chl. + 1.23 μg/cc nicotinic acid	39	43	80	42

*A = Inoculum of 300,000 organisms per cc.
†B = Inoculum of 300 organisms per cc.
‡5.84 μg of cozymase contained 0.0012 μg of thiamin chloride.

same in the two tubes. The lag in initial growth with thiamin chloride and nicotinic acid is very likely due to the fact that nicotinic acid must first be converted into cozymase by the bacterial cells before the growth requirements for bacterial reproduction can be fulfilled.

The second group of studies concerned the comparative effect of thiamin chloride and nicotinic acid, and cozymase upon the bacteriostatic action of sulfanilamide, sulfapyridine, sulfathiazole, and sulfadiazine in the presence of a large and small inoculum of organisms. The results with a large inoculum (500,000 organisms per cc) are shown in Table II. Under these conditions, bacterial growth was practically the same in the tubes containing cozymase, and thiamin chloride and nicotinic acid. A slight inhibitory effect was produced by sulfathiazole.

The results with a small inoculum of organisms (3500 organisms per cc) are presented in Table III. When the tubes were examined at the end of 43 hours of incubation, the bacteriostatic effect of sulfanilamide was inhibited in the tube containing cozymase, but not

TABLE II.
Comparative Effect of Thiamin Chloride, Nicotinic Acid and Cozymase upon Bacteriostatic Action of Sulfonamide Compounds.
(Inoculum approximately 500,000 organisms per cc.)

	Thiamin Chloride, μg/cc	Nicotinic Acid, μg/cc	Cozymase,† μg/cc	Growth in % of light transmitted at 20 hr
Basal Medium	—	—	—	68*
	—	1.23	—	47
	.034	1.23	—	31
	—	—	9.33	32
Basal Medium +	—	—	—	70
Sulfanilamide	—	1.23	—	55
50 μg/cc	.034	1.23	—	38
	—	—	9.33	32
Basal Medium +	—	—	—	70
Sulfapyridine	—	1.23	—	46
50 μg/cc	.034	1.23	—	31
	—	—	9.33	36
Basal Medium +	—	—	—	68
Sulfathiazole	—	1.23	—	54
50 μg/cc	.034	1.23	—	45
	—	—	9.33	40
Basal Medium +	—	—	—	69
Sulfadiazine	—	1.23	—	50
50 μg/cc	.034	1.23	—	34
	—	—	9.33	38

*Medium initially slightly cloudy but sterile.
†9.33 μg cozymase contain 0.0019 μg thiamin.

in the tube containing thiamin chloride and nicotinic acid. When duplicate tubes with sulfanilamide were examined at the end of 87 hours, the degree of growth was the same in the tube containing cozymase as in the tube containing thiamin chloride and nicotinic acid. No inhibition of the bacteriostatic effect of sulfapyridine, sulfathiazole or sulfadiazine was exhibited.

A similar experiment was carried out in which the original inoculum was only 60 organisms per cc. When the tubes were examined at the end of 140 hours of incubation, sulfanilamide and sulfapyridine had failed to inhibit the growth of organisms in the tubes containing cozymase, but a complete bacteriostatic effect was exhibited in the tubes with thiamin chloride and nicotinic acid. This difference was not apparent at 72 hours, both sets of tubes showing complete inhibition at this time.

Comment. The ability of the cozymase preparations used in these experiments to replace both thiamin and nicotinic acid is probably due to a contamination of the cozymase with thiamin.

TABLE III.
Comparative Effect of Thiamin Chloride, Nicotinic Acid and Cozymase upon Bacteriostatic Action of Sulfonamide Compounds.
(Inoculum approximately 3500 organisms per cc.)

	Thiamin Chloride, μg/cc	Nicotinic Acid μg/cc	Cozymase, μg/cc	Growth in % of light transmitted at 43 and 87 hrs	
				43 hr	87 hr
Basal Medium	—	—	—	99	100
	—	1.23	—	83	89
	.0034	1.23	—	49	50
	—	—	5.84	47	50
Basal Medium + Sulfanilamide 50 μg/cc	—	—	—	100	98
	—	1.23	—	96	100
	.0034	1.23	—	95	43
	—	—	5.84	52	49
Basal Medium + Sulfapyridine 50 μg/cc	—	—	—	96	98
	—	1.23	—	97	98
	.0034	1.23	—	97	96
	—	—	5.84	92	87
Basal Medium + Sulfathiazole 50 μg/cc	—	—	—	100	97
	—	1.23	—	98	96
	.0034	1.23	—	100	100
	—	—	5.84	96	98
Basal Medium + Sulfadiazine 50 μg/cc	—	—	—	98	96
	—	1.23	—	97	98
	.0034	1.23	—	96	98
	—	—	5.84	97	93

5.84 μg cozymase contain .0012 μg thiamin.

A small number of organisms in the presence of thiamin chloride, nicotinic acid and sulfanilamide or sulfapyridine show a lag period in growth. On the other hand, when cozymase is present, growth becomes more readily established. We have assumed that cozymase must be synthesized by the cells before reproduction takes place. Through a mechanism not clearly understood either sulfanilamide or sulfapyridine will interfere with this synthesis. Sulfathiazole and sulfadiazine in the concentration used in these experiments showed complete bacteriostasis for a small number of organisms even in the presence of cozymase. These results confirm the observations of West and Coburn.

When a large number of organisms are used, they are apparently able to synthesize cozymase so rapidly that there is no difference in the degree of growth obtained whether nicotinic acid or cozymase is present. The possibility still remains that the lag period produced by the sulfonamides may be shorter when cozymase is present than when nicotinic acid supplies the growth stimulus. This should be investigated. The bacteriostatic activity of sulfathiazole and sulfadiazine is not completely inhibited by either cozymase or nicotinic acid.

It has been demonstrated by many investigators that the bacteriostatic effect of the sulfonamide compounds is more pronounced when the optimum requirements for bacterial growth are lacking and the number of organisms is small. The foregoing observations are in accord with this concept.

Summary. 1. Two samples of cozymase were tested, and each could replace both thiamin chloride and nicotinic acid, essential growth requirements for the staphylococcus; however, these preparations contained small quantities of thiamin. It has been shown that an adequate amount of thiamin was contained in the cozymase, which explains why maximum growth of staphylococci was obtained in the presence of cozymase, but in the absence of nicotinic acid. 2. When certain experimental conditions are fulfilled, cozymase, but not thiamin chloride and nicotinic acid, will inhibit the bacteriostatic action of sulfanilamide and sulfapyridine against staphylococci.