ponent was observed. The sedimentation constants corrected to water at 20° are shown in Table III. The average of the 6 values

TABLE III.
Sedimentation Data on Infectious Material from Diseased Silkworms.

Sample	s^{20}_w Svedberg units	Sample	s^{20}_w Svedberg units
A	17.7	D	19.9
B	17.9	D*	16.8
C	14.4	E	16.4

* After 3 months' storage at 4°C.

reported in Table III is about 17 S. This figure is obviously not significantly different from that obtained for the homogeneous constituent of normal fluid. The material it represents must be, of course, the more rapidly sedimenting constituent of the undiluted blood of diseased worms.

The components found in this study to be present in the blood of both healthy and diseased worms are entirely different from the 2 components extracted by Glaser and Wyckoff[8] from the frozen and ground bodies of both healthy and jaundice-diseased silkworms. The blood components have corrected sedimentation constants of 16 or 17 S and about half of that value; the extracts of whole worms showed 2 components with corrected sedimentation constants of 53 and 92 S. The blood constituents are extremely stable, for they are apparently the same in fresh blood and in blood stored in the cold for 3 years; the components extracted from whole worms disintegrate within a few days.

Summary. The blood of normal and of jaundice-diseased silkworms and a highly infectious purified material isolated by Glaser and Stanley[5] from the blood of diseased worms were studied in the ultracentrifuge. No difference between the bloods of healthy and diseased worms was observed by this method. Both contained a homogeneous component with a corrected sedimentation constant of 16 or 17 S and a very inhomogeneous, more slowly sedimenting component. The only component present in an optically detectable amount in the highly infectious purified material was found to have a corrected sedimentation constant of about 17 S.

[8] Glaser, R. W., and Wyckoff, R. W. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 503.

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Inhibition of Bacterial Growth by Glucose in Media Devoid of Nicotinic Acid.

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In preceding publications[1] it was shown that *Sal. paratyphi* A. and *Sh. dysenteriæ* give appreciable growth in a synthetic or semi-synthetic medium in the absence of nicotinic acid, provided that no sugar or alcohol fermented by these organisms is present. The addition of glucose to such a substrate inhibited growth, while the non-fermentable sucrose (or lactose) had no such unfavorable effect.

The present paper presents data showing that this inhibitive effect obtains with other bacteria requiring nicotinic acid for their utilization of carbohydrates and related substances. In these experiments we tested *B. proteus* XK, three strains of *Sh. dysenteriæ* (Schmitz, Flexner and Hiss), and *Staphylococcus aureus.*

The proteus strain was grown in a medium consisting of the salt solution recommended by Fildes (without glucose or nicotinic acid),

[1] Fildes, P., *Brit. J. Exp. Path.*, 1938, **19**, 240; Kligler, I. J., and Grossowicz, N., *J. Bact.*, 1939, **38**, 309; Kligler, I. J., and Grossowicz, N., *Nature*, 1940, **146**, 652; Kligler, I. J., and Grossowicz, N., *J. Bacteria*, 1941, **42**, 173; Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 731.

TABLE I.
Relative Growth of Proteus and Dysentery Organisms in Various Media with and without Nicotinic Acid.

	Proteus XK Synthetic medium		Proteus XK Casein hydrolysate		Flexner X		Schmitz Casein hydrolysate		Hiss	
	Growth density	Plate count (millions)	Growth† density	Plate count	Growth density	Plate count	Growth density	Plate count	Growth density	Plate count
Without nicotinic acid:										
Basic medium	++	180	88	124.6	90	32	87	120	88	116
" and glucose	+	42	97	10.8	99	0.2	100	0.01	99	0.16
" " lactate	++	140	92	62.6	87	123	88	72	86	200
With nicotinic acid:										
Basic medium	++	590	72	400	78	330	76	370	75	390
" and glucose	+++	620*	68	540	59	1,520	54	1,860	59	1,430
" " lactate	+++	1,260	66	580	70	690	70	698	67	980
Sterile control	—									

* Many organisms died as a result of the high acidity.
† Photometer readings; sterile tubes = 100.

to which was added 0.3% of an acid hydrolysate of glaxo "ashless" extracted casein: (semi-synthetic medium), or 0.25% asparagine, 0.05% cystine and 0.5% glycine: (synthetic medium).

The dysentery organisms were grown in the following medium: A. Salt solution: NaCl 5.0 g; Na_2HPO_4 2.5 g; K_2HPO_4 0.35 g; H_2O 900 ml; this solution was divided into tubes (4.5 ml per tube) and autoclaved; B. To each tube was added 0.1 ml of a 4% solution of $MgSO_4$ and 0.3 cc of a 10% casein hydrolysate. For staphylococci 0.01% tryptophane was added to this medium.

Parallel growth tests were made in substrates with and without the addition of nicotinic acid (1-2 γ per ml), both in the presence and in absence of glucose (0.1%-0.2%). In the case of staphylococci, which require both nicotinic acid and thiamine, all combinations were tested. The tubes were sown with 1000-4000 washed organisms and incubated in a slanting position for 4 days at 37°C. At the end of that time plate counts and photometric turbidity readings were made. The results are summarized in Tables I and II.

The results show that in the synthetic amino acid medium, in the absence of nicotinic acid, the proteus and dysentery organisms grow 5 to 1000 times more abundantly when no glucose is added than they do in its presence. The staphylococcus grows only sparsely in this medium, but such growth as there is is 100-1000 fold greater in the absence of than in the presence of glucose. It is clear, therefore, that in a nicotinic acid-free medium glucose has an inhibitive effect on growth.

All available evidence indicates that the

TABLE II.
Relative Growth of *Staph. aureus* in Various Media, with and without Nicotinic Acid.

	Basic medium		Basic + 0.2% glucose		Basic + 0.1% lactate		Basic + 0.1% pyruvate	
Medium	Growth density	Count*	Density	Count	Density	Count	Density	Count
Without nicotinic acid:								
+ Thiamine	95	0.95	99	0.006	96	0.13	90	1.10
— Thiamine	98	0.075	100	0.0008	99	0.015	96†	0.095
With nicotinic acid:								
+ Thiamine	60	410.0	49	2,750	57	864.0	55	1,230.0
— Thiamine	79	27.6	65	160	70	97.0	69	103.0

* In millions of colonies per ml.
† The color of the pyruvate influences the photometer readings.

presence of glucose interferes with the oxidation of the amino acids. The quantitative differences in the effect on different organisms, as well as the variable influence of lactic acid, must be presumed to be due to the different capacity of each organism for aerobic oxidation of the hydrocarbons and amino acids. The stimulating effect of nicotinic acid in the amino acid medium without carbohydrates can also be explained on this basis. A more detailed study of the relation of vitamins to the metabolic activity of staphylococci (to be reported elsewhere) indicates that the oxidation of amino acids is similar to that of lactate and pyruvate.

Summary. When nicotinic acid is lacking in a substrate otherwise suitable for growth, the addition of glucose inhibits the growth of organisms capable of fermenting this sugar in the presence of nicotinic acid. The degree of inhibition is 5 to 1000 fold, and depends on the organism and the medium. With Proteus XK the inhibition was 5-10 fold; with dysentery organisms and with staphylococci 100-1000 fold.

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Effect of Glutamic Acid on the Central Action of the Ammonium Ion.

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In 1936, in a study of the effect of various stimulants and depressants of the central nervous system on the aftercontraction in humans, it was noted that small amounts of ammonium salts (.5 g ammonium), taken by mouth, caused a marked increase in this phenomenon. We have considered the aftercontraction a measure of the excitability of the human cerebral cortex.[1,2,3] It has been repeatedly affirmed in the literature that the concentration of ammonium ion increases in the brain tissue and cerebrospinal fluid in states of increased excitability of humans and animals. The fact that oral and parenteral administration of ammonium salts can produce convulsions in animals is well known.

Since ammonium convulsions so closely simulate spontaneous seizures, it seemed that any substance which would prevent these seizures, might be useful in other types of convulsive activity. The work of Krebs on the metabolism of amino acids in brain tissue slices offered an important clue.[4] Of many substances tested he found that glutamic acid specifically caused a marked reduction in the ammonium ion concentration present in the medium surrounding the tissue slices, due to the action of the enzyme glutaminase which synthesized glutamine from glutamic acid and ammonia. The enzyme system was reversible, was stimulated by the physiological *l* (+) glutamic acid and inhibited by *d* (—) glutamic acid. Under this concept, it should be possible to increase or decrease the ammonium ion concentration of the brain and thus control its convulsive activity. Experiments to test this hypothesis were begun in July, 1942.

In our studies on the role of ammonium ion in the convulsive state, we developed a uniform method of production of seizures in rabbits by the slow intravenous injection of 2½% ammonium chloride solution. One cc per kilo of this solution is administered every 5 minutes until a generalized convulsion results. All 12 of the rabbits (2 kg) used in these experiments had convulsions after 15-25 cc of the solution had been injected. The observations were

[1] Sapirstein, M. R., Herman, R. C., and Wallace, G. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 163.

[2] Sapirstein, M. R., Herman, R. C., and Wallace, G. B., *Am. J. Physiol.*, 1937, **119**, 549.

[3] Sapirstein, M. R., Herman, R. C., and Wechsler, I. S., *Arch. Neurol. and Psychiat.*, 1938, **40**, 300.

[4] Krebs, H. A., *Biochem. J.*, 1935, **29**, 1951.