10 animals that survived showed evidence of meningitis when they were sacrificed.

The site of the inoculation of the organism into the rabbits determines the course of the disease process. If small quantities of organisms are inoculated into the cisterna, the rabbits died within 24 to 48 hours with an extensive meningo-encephalitis. On the other hand, intramuscular or intraperitoneal injections did not produce any apparent change, either clinically or anatomically.

Mice, guinea pigs and young monkeys were found to be susceptible to intravenous inoculation of the organisms.

In summary, there has been isolated from the blood stream of 3 newborn infants an unidentified gram positive bacillus that is found to be pathogenic for the usual laboratory animals and apparently has a predilection to localize in the central nervous system when given intravenously in small quantities.

7453 C

Validity of Iodine and Copper Reduction Methods for Amylase.

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If a quantitative method for the determination of amylase be valid and practical, different samples of the substrate—soluble starch—must give similar and reproducible results.

In a previous paper[1] the Wohlgemuth and viscometric methods were investigated by comparing the amounts of enzyme required to produce a given result in different samples of soluble starch. Both of these methods measures the dextrinogenic constituent of amylase; the Wohlgemuth was found to be non-valid as a quantitative procedure, while the viscometric method seems to be valid if properly controlled. Thompson, McGarvey and Wies[2] have obtained good results with the viscometric method by mixing together different samples of soluble starch.

The purpose of the present paper is to investigate the iodine and copper reduction methods as indicated above; the latter method is for the measurement of the saccharogenic agent of the amylase complex.

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1 Chesley, L. C., *J. Biol. Chem.*, 1931, **92**, 171.

2 Thompson, W. A., McGarvey, S. M., and Wies, C. H., *J. Gen. Physiol.*, 1932, **16**, 229.

A. The achromic iodine method and variations.

The first procedure used was that devised by Johnson.[3] The determinations were conducted exactly as specified in his paper; no sodium chloride or phosphate buffer were used. The substrate was a 2% soluble starch preparation; correction was made for the moisture content.

Into each of a series of flasks in a water bath at 40°C. were placed 50 ml. portions of the soluble starch, which was allowed to come to the temperature of the bath. At 10 second intervals, successively increasing amounts of dilute saliva were added to the flasks. (Different specimens of human saliva diluted with different volumes of distilled water were used.) At the end of 10 minutes, 5 drops were removed from the digest and added to 5 ml. of N/8000 iodine solution. The first tube in which no color was developed was taken as the end-point. The quantity of enzyme solution which would just digest the starch to the end-point was determined as accurately as was practicable. The figures given in Table I under "ml. enzyme solution" represent the quantity of dilute saliva required to hydrolyze the soluble starch to the end-point in 10 minutes.

Two soluble starch samples were used simultaneously and under identical conditions. Sample 1 was Baker's analyzed C. P. soluble starch; Sample 2 was Lintner's soluble starch.

As shown in Table I, Series 1, Sample 1 required about 25% more enzyme than did Sample 2 to be digested to the same end-point in the same time. In Series 2, 3, and 4 (Table I) the effects of added chloride and phosphate buffer, both separately and together, are shown. The added sodium chloride was 0.05 M in final concentration, as was the phosphate buffer mixture, the pH, in the buffered series, was 6.9.

In all 3 cases, Sample 2 required about 45% more enzyme to be digested to the same end-point as Sample 1. It is seen that the relative digestibility is the reverse of that in the first series in which no electrolyte was added. The effect is not caused by a pH change, since Series 3 shows the same reversal as do Series 2 and 4. It may be caused by the presence of a greater quantity of activating electrolyte impurity in Sample 2.

Phosphates or chlorides, present as impurities in different amounts in the samples, are not responsible for the discrepancy in "digestibility" which exists between the 2 samples, since in Series 4 enough

[3] Johnson, W. A., *J. Am. Chem. Soc.*, 1908, **31**, 798.

TABLE I.

Digestion of soluble starch Samples 1 and 2, as influenced by added electrolytes. Six experiments with different specimens of saliva. Disparity in "digestibility" of samples indicated by quantities of enzyme solution required for hydrolysis to end-point.

Exp.	Series 1 Unbuffered (1), pH 6.5; (2), pH 6.0 Added phosphate, none Added NaCl, none Ml. enzyme solution Sample 1	Sample 2	Ratio	Exp.	Series 2 Buffered pH 6.9 Added phosphate, .05 M Added NaCl, none Ml. enzyme solution Sample 1	Sample 2	Ratio
1	1.20	1.00	1.20:1	1	1.20	1.60	.75:1
2	1.20	1.00	1.20:1	2	1.00	1.60	.62:1
3	0.80	0.60	1.33:1	3	1.10	1.50	.73:1
4	0.80	0.60	1.33:1	4	0.80	1.20	.67:1
5	0.80	0.60	1.33:1	5	0.80	1.20	.67:1
6	0.80	0.70	1.14:1	6	0.70	1.00	.70:1
	Mean		1.25:1		Mean		.69:1

Exp.	Series 3 Unbuffered (1), pH 6.5; (2), pH 6.0 Added phosphate, none Added NaCl, .05 M Ml. enzyme solution Sample 1	Sample 2	Ratio	Exp.	Series 4 Buffered pH 6.9 Added phosphate, .05 M Added NaCl, .05 M Ml. enzyme solution Sample 1	Sample 2	Ratio
1	.40	.65	.61:1	1	.15	.225	.67:1
2	.40	.60	.67:1	2	.20	.275	.72:1
3	.40	.60	.67:1	3	.15	.250	.60:1
4	.40	.60	.67:1	4	.20	.250	.80:1
5	.40	.60	.67:1	5	.20	.300	.67:1
6	—	—	—	6	.30	.400	.75:1
	Mean		.65:1		Mean		.70:1

of both chloride and phosphate was added to render negligible the differences in concentrations of these contaminations.

B. The copper reduction method.

In the present investigation, the Walker-Munsen and Bertrand methods were combined for the estimation of maltose (Mathews[4]).

Two percent soluble starch was prepared, sodium chloride and phosphate buffer was added to 0.05 M each, and the pH was maintained at 6.9; the temperature was held at 35°C. To 25 ml. portions of the soluble starch was added one ml. of diluted saliva. At the end of 30 minutes, 5 ml. specimens were removed from the digest and run into an excess of Fehling's solution (25 ml. diluted to 95 ml.). The solution was kept in a bath at 112°C. for exactly 6 minutes, then removed and filtered through a Gooch crucible using suction. The precipitate was washed several times with distilled water at 60°C., then dissolved in M/2 ferric ammonium sulphate-sulphuric acid mixture and titrated at once with N/20 potassium

[4] Mathews, A. P., Physiological Chemistry, 1920, 3d ed., p. 891, New York.

permanganate. Two starch samples were compared at once. The results are shown in Table II. It will be seen that the 3 soluble starch samples and dextrin (Merck's "Blue Label") are saccharified at the same rate.

TABLE II.

Comparison of soluble starch Samples 1, 2, and 3 with dextrin. "Digestibility" as determined by the copper reduction method.
Starch 2%. pH 6.9. Temp. 35° C. NaCl .05 M. Phosphate .05 M.
Six experiments with different specimens of saliva.

Sample Exp.	Mg. maltose produced per ml. in 30 minutes a	b	c	d	e	f	av.	Variation %
1	14.0	11.2	9.7	12.7	13.6	13.2	12.4	
2	14.3	11.8	10.8	12.8	13.4	13.2	12.7	2.5
2	11.0	11.9	10.9	12.9	11.9	10.9	11.6	
3	11.0	12.1	10.7	13.0	12.4	11.3	11.7	1.12
3	11.1	11.5	10.2	11.1	11.6	12.4	11.3	
Dextrin	11.0	11.5	10.2	11.8	11.5	11.9	11.3	0.0

Soluble starch is prepared by incipient acid hydrolysis of starch, and by other methods not in commercial use. One source of error in the older experiments with amylase determination has been the differences in chloride concentrations in different samples; another has been acidity. In modern methods, these are eliminated by the addition of relatively large amounts of chloride and buffer salts. However, as shown here, there are other errors which are apparently caused by differences in the chemical constitutions of different soluble starch samples.

As shown above, the copper reduction method yields very closely comparable results from sample to sample of soluble starch and dextrin; the enzyme determined by this method has dextrin as its substrate (possibly also soluble starch). The iodine methods give widely discrepant values. It has been pointed out (Chesley[1]) that the order of relative digestibility of 3 soluble starch samples as measured by the viscometric method is the reverse of the order as determined by Wohlgemuth's iodine method. If the viscosity of soluble starch is caused by amylopectin, as Samec[5] believes to be the case for starch, then the viscometric method measures primarily the hydrolysis of amylopectin. As for the iodine methods, that of Wohlgemuth depends upon the complete disappearance of amylose (and other substances giving a blue iodine reaction), while the achromic methods depend upon the total disappearance of all substances giving color reactions with iodine. If the concentrations of such substances vary from sample to sample of soluble starch, then

[5] Samec, M., Comprehensive Survey of Starch Chemistry, 1928, 51, New York.

different amounts of amylase will be required to hydrolyze the digest to the end-point in a given time.

From the evidence adduced in this and a previous paper (Chesley[1]), it seems probable that the disparity in "digestibility" (as given by the various methods) is caused by varying proportionate quantities of amylopectin, amylose and dextrins in the different soluble starch samples.

Summary. The achromic iodine method, with several variations, has been investigated for its validity in determining amylase.

The iodine method is not a valid quantitative procedure for the estimation of amylase. There are considerable differences in the quantities of enzyme required to digest different samples of soluble starch to the same end-point in the same time, under identical experimental conditions.

The copper reduction method is a valid quantitative method for measuring the saccharogenic power of an amylase. Dextrin and 3 samples of soluble starch were found to be saccharified at the same rate.

Apparently the disparities in digestibility which exist among different soluble starch samples are caused by varying relative amounts of amylopectin, amylose and dextrins.

7454 P

A Method for Obtaining Stable S Colonies of Human Tubercle Bacilli.

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The so-called "S" colonies of the human tubercle bacillus obtained by Petroff,[1] Reed and Rice,[2] and others, as described or shown on photographic plates, although more easily emulsified in saline and of greater virulence than R colonies, appear flat, wrinkled, dull, and irregular rather than convex, glistening, round, and smooth-edged as do S colonies of bovine and avian tubercle bacilli, and the S colonies of most other bacteria which have been dissociated. The flat colonies seem to be intermediates and not true S. This paper reports a successful attempt to dissociate human tubercle

[1] Petroff, S. A., and Steenken, W., Jr., *J. Exp. Med.*, 1930, **51**, 831.

[2] Reed, G. B., and Rice, C. E., *Canad. J. Res.*, 1931, **4**, 389; **5**, 111.