Type I

Soluble Specific Substance 29

OCT 30 1923 ff. — Lots 14, 15, 16, bile soln. & washings from organisms from l. culture filtered separately into 1 l. bottles, and 10 g. $Al(OH)_3$ added to ea. after acidifying w. 5 cc. HOAc. Addn. of $Al(OH)_3$ cont'd. until supernatant —, then sucked off, ppt. washed w. H_2O, v extd. liv. 1%, then 2% Na_2HPO_4 until ext. —. Exts. neutr. w. HOAc & concd. to sm. bulk on H_2O bath. Some bacterial contamination during long standing.

1 Ads.

FEB 8 1924 — Lots 18 & 22 started in same way.

MAR 5 — Lots 23 & 24 " " " ".

83.5 liters

2 APR 3 — Concentrates decanted, rest centfgd., & at vol. of ca. 2 l., pptd. w. 3 l. alc. APR 4 — Supernatant, —, sucked off, ppt. centfgd., sepg. into upper clear layer, lower aq. layer satd. w. salts coated w. S.S. Aq. portion of lower layer —, discarded. APR 5 — Ppt. diss.

3 in ~~[illegible]~~ H_2O, centfgd., HOAc added. At vol. of 300 cc., pptd. w. 400 cc. alc. APR 7 — Supernatant —, sucked off, ppt. centfgd., again sepg. into more of top layer, middle gummy layer, & bottom aq. salt layer: Last also —, so discarded. Ppt. diss. in ca. 80 cc. H_2O, centfgd. APR 8 — With washings, = ca 100 cc. 25 cc. alc. added, pptg. part of active material. Left in ice-box in stoppered flask.

4 SEP 27 — Heavy ppt., but supernatant +. ~~50~~ 75 cc. more alc. added. SEP 29 — Supernatant almost —, sucked off. Ppt. centfgd. Middle layer diss. in hot H_2O & very viscous soln. concd. dry on H_2O bath. ~~[illegible]~~ Diss. in hot H_2O, centfgd. OCT 1 — Soln. again concd. dry. Diss. in hot H_2O, centfgd. OCT 2 — At vol. of ca. 40 cc., 10 cc. 1:1 HCl added, subst pptd. w. 70 cc. alc. Supernatant +, so 30 more alc added.

5 ~~OCT 3~~ Centfgd. in cold. Supernatant, weakly + made faintly alk. w. 10% NaOH, giving mainly crystn. ppt. containing the active material. Added to 36. Main ppt. diss. in H_2O, neutr. w. NaOH. OCT 3 — Soln. made slightly acid w. HCl, centfgd. Run through Berkefeld V, but evidently acted on filter, as considerable flocculent material sepd. in filtrate. Washed through w. H_2O, neutr., filtered. OCT 4 — Concd. to ~~[illegible]~~ dryness. OCT 7 — Centfgd. from insol. material after taking up in hot H_2O. OCT 8 — Again concd. dry, taken up in hot H_2O, centfgd., ppt. washed. OCT 9 — At vol. of 20 cc., made alk. w. 1 cc. 10% NaOH,

6 pptd. w. 40 cc. alc. OCT 10 — Supernatant, —, sucked off, ppt. centfgd., diss. in H_2O. Viscous soln. treated w. N HCl until permanent ppt. Acid to litmus, not to Congo. Centfgd. in the cold. Few more drops N HCl gave more ppt. Again centfgd. No further ppt. in test portion of supernatant. p_H of main supernatant, by drop method using bromcresol green, 4.1. Supernatant (A) decanted, ppt. washed w. ca 10 cc. H_2O, centfgd.

Supernatant (A_2) decanted, centfg. ppt. again to get sharp sepn.
A ++ w. serum, A_2 ++++, ppt. B, ~~fairly~~ sl. in saline, soln. +++, gives Molisch, Ppt. redissolves w. xs. HCl.

OCT 11 – Ppt. taken up in ca 10 cc. H_2O + 1 drop 25% HOAc, smoothed out, again centfgd. Supernatant A_3 ++++. ~~All sol~~ A_1, A_2, A_3 combined & diald. in collodion bags agst. distd. H_2O. B. suspended in a little H_2O, N HCl added until redissolved. Viscous soln. rinsed into collodion bag & diald. agst. distd. H_2O, w. frequent changes.

OCT 14 – Mixt. from which B had reppld, centfgd, supernatant added to A. Ppt. washed w. alc., acetone, removing trace of coloring matter & sl. activity. Yield: 0.185 g.

? Active agst. type I serum at dilu. of 1:500,000

Tests on 1:200 Soln. 29 B

	Biuret –	Negative
	Tannic Acid –	"
Rejected	I-KI –	"
Jun 22-3.	HPW –	Heavy ppt., insol. w. xs. N HCl
Active	$CuSO_4$ –	Gelatinous ppt.
at	$Hg H_2(SO_4)_2$ –	
1:4,000,000	$AgNO_3$ –	Heavy ppt.
(highest tested)	$Pb(OH)OAc$ –	Gelatinous ppt.
	$Pb(OAc)_2$ –	~~Gelatinous ppt.~~
	Fehling –	Negative before hydrolysis, reduction after hydrolysis.
A & B	Millon Reagt. –	Gelat. ppt. in cold. Diss. on hdtg. Test –.

probably got reversed in this test the first time.

A centfgd., soln. concd. to dryness, finally in Pt dish.
Yield: 0.0688 g.

? Active agst. type I serum at dilu. of 1:3,000,000 (highest tested).

Biuret	–	Negative
Tannic Acid	–	"
HPW	–	Heavy ppt., insol. w. xs. HCl.
$CuSO_4$	–	Gelatinous ppt.
$AgNO_3$	–	Heavy ppt.
I-KI	–	Negative.
$Ba(OH)_2$	–	Opalescence

S. S. S. 29 A

OCT 24 – Wt. dish + subst ⊗ 23.4954 23.4943 Susp'd. in H_2O, 3 drops
Wt. dish 23.4255 N NaOH necessary for clear
.0688 soln., made up to 10 cc.

OPTICAL ROTATION

⊗ = +0.01 -02 -02. Rdgs. +1.56 1.57 1.57 l = 1

$$[\alpha]_D = \frac{155}{.0688} = +225°$$

$[\alpha]_D = +225°$

Microkjeldahls

Standard: 0.5 mg. N in 150 cc. 2 cc. soln.
" 55 Unknown in 50 cc.
Rdgs: 32.8 31.3 32.3 31.6 30.5 5 | 158.5
Mean: 31.7

In 50 cc. Standard 0.167 mg. N In soln. $\frac{55}{31.7} \times .167 = .29$ mg. N

In 10 cc. soln. 1.45 mg. N = 2.1%

N 2.1%

Hydrolysis

OCT 28 2 cc. dil'd. to 4, b. 5 hrs. w. 4 cc. 2N HCl, neutr. w. Na_2CO_3, made up to 10 cc.

1.5 cc. samples.

cc. $Na_2S_2O_3$	I	II	Blank
	15.85	31.60	19.4
		15.90	15.8
		15.70	3.6 cc.

Mean: 15.8

3.6 × 1.016 × .318 = 1.16 mg. Cu
= 0.6 " glucose

$$\frac{0688}{5} \times \frac{1.5}{10} = 2.06 \text{ mg. subst.}$$

Redg. Sugars
30%

S. S. S. 29 B

OCT 15 1924 Wt. bot (3) + subst. ~~7.3800~~ 7.4043 7.4035
7.3028
.1007

Suspd. in H_2O, diss. w. 4 drops N NaOH, made up to 20 cc. Soln. neutr. to litmus

OPTICAL ROTATION

Bl. +0.03 0.02 .01 .01 .02 $l = 2$

Rdgs. +3.05 3.05 3.05 Dr. Jacobs: 3.04

$[\alpha]_D = \frac{303}{1007} = +301°$

$\boxed{[\alpha]_D = +301°}$

Soln: dil'd. w. = vol. N HCl $l = 2$

0 = 0

Rdgs. 1.31 1.62 1.53 1.46 1.47 1.49

$[\alpha]_D = 150 \times 2 = +300°$

Microkjeldahls · 1 cc. sample

	I		II		Bl.		
cc. N/50 HCl (×1.006)	2.00		2.00		2.00		
cc. N/100 NaOH (×0.997)	10.10		12.22		11.89		
	8.00		10.12		8.00		
~~Calc. as N/100 HCl~~	2.10		2.10		3.89	~~4.02~~	Goebel's Blank. 0.18
Calc. as N/100 HCl		4.02		4.02		4.02	Mean:
N/100 NaOH		2.09		2.09		3.88	0.16
		1.93		1.93		.14	
				.16			
				1.77			

1 cc. sample cont. 0.0000248 gm. N
In 20 cc. .0000496 " N

$\boxed{\text{Total } N = 5.00\%}$

HYDROLYSIS

OCT 20 3 cc. sample dil'd. to 6 cc., 6 cc. N HCl added, b. 5 hrs. Set stand in ice box over night, neutr. w. Na_2CO_3, made up to 20 cc.

OPTICAL ROTATION

Bl. 0 = +0.01 0.00 Rdgs. +0.38 0.38 0.42 0.42

Specificity: ++++. Redg. sugars: Very slight, showing practically none of subst. hydrolyzed.

To 15 cc. soln. 3 cc. conc. H_2SO_4 added, b. again. [redg. sugars Turned dark, showing charring. Abnormal vals. for

Evidently basic N had Na of Na salt had combined w. much of free HCl.

Discarded.

S.S.S. 29 B

Amino N Detns.

OCT 20	t	Barom.	Rdg.	Corr.	Mean
Bl. 1	22.5°	759.6	0.105	0.104	0.100
	22°		0.098	0.097	
		2 cc.	1:400	soln. used.	Bl.
1	22.5°	760	0.327	0.326	0.226
2	21.5°		0.350	0.349	0.249

In 100 cc. soln. 250 mg.

∴ % NH_2 N 2.5%, 2.8%

$\frac{.226 \times .5623 \times 100}{2} = 6.36$ mg. NH_2 N per 100 cc.

$\frac{.249 \times .5650 \times 100}{2} = 7.04$

NH_2 N 2.7%

HYDROLYZED SOLUTION

1.	24°	768	0.210	.209	0.109
2.	23.5		.218	.217	0.117

12.5 mg. substr. in 15 cc., 83.3 mg. in 100 cc.

$\frac{.109 \times .109 \times .5640 \times 100}{2} = 3.07$

$\frac{.117 \times .5655 \times 100}{2} = 3.31$ mg NH_2 N per 100 cc.

Mean: 3.19

NH_2 N after HYDROLYSIS 3.8%

HYDROLYSIS

OCT 22 — 3 cc. 1:200 soln. dil. to 6 w. H_2O, 6 cc. 2N HCl added. B. 6 hrs.

Bl.: +0.04 .02 .03 Rdgs. + 0.13 .13 13. 5 cm. tube

$[\alpha]_D = +160°$

B. 1 hr. longer.

Bl. +0.01 0.00 0.00 Rdgs. + 0.11 .10 .10

$[\alpha]_D = +160°$

10 cc. made up to 15, neutr. w. Na_2CO_3, 4 cc. taken for redg. sugar detns by Schaffer micro method.

cc. $Na_2S_2O_3$	11.80	23.50
		11.80
		11.70

Mean: 11.75

Blanks: (Goebel) 19.00 − 11.75 = 7.25 cc.

$7.25 \times 1.016 \times .318 = 2.343$ mg. Cu = 1.06 mg. glucose

$\frac{12.5 \times 4}{15} = 3.333$ wt. of substr. in sample

Redg. Sugars calc. as glucose 31.8%

JAN 13 1925 — ~~C & H by Dr. Eleck.~~

~~0.1020 g. subst. gave 0.0524 g. H_2O & 0.1594 g. CO_2~~

~~C = 42.61% H = 5.74%.~~

Jun. 20. Repetition of N (distn).

Aft. bot 11 + substr 8.3240 made up to 11.1 cc. 5 cc. for anal.

8.2870

.0370

cc. N/70 HCl (f 8.95)	15.00	15.00	14.92
+ N/70 Na_2CO_3 (f 1.015)	10.60	10.60	10.76
			4.16
			11
			4.05

mg. N 0.81

% N 4.8

Total N 4.8%

Type I.

Soluble Specific Substance 32

JAN 18 1924 — 3 l. day old culture of I-193-18 in Pn broth concd. on H_2O bath to ca. 200 cc. Pptd. w. 250 cc. alc. JAN 19 — Dark supernatant —, sucked off., ppt. centfgd., giving usual 3-layer sepn. Middle layer & washings diss. in H_2O to 50 cc., centfgd., & clear soln. pptd. w. 60 cc. alc. JAN 21 — Upper layer, —, sucked off, ppt. centfgd., & cake sepd. from top & bottom layers Diss. in little H_2O, acidif. w. HOAc, centfgd. Clear soln (30 cc.) + 10 cc. alc. turbid. Centfgd. Clear supernatant + 40 cc. alc. — heavy ppt. After several hrs., test of supernatant, centfgd., —. Whole centfgd. JAN 22 — Ppt. diss. in 15 cc. H_2O, 5 cc. N NaOH added, followed by several drops 10% NaOH until max. ppt. Centfgd. Clear supernatant pptd. w. 30 cc. alc. Centfgd. after ca. 2 hrs. Supernatant —, ppt. taken up in ca 10 cc. H_2O, almost neutr. to litmus w. N HCl, rinsed into centfg. tube w. 2 cc. H_2O, centfgd., poured into 125 cc. redistd. acetone. JAN 23 — Ppt. sucked off, washed w. acetone, sucked dry, dried at 100°, then in vac. Yield (contg. salts): 0.6 g.

Activity very low, apparently mainly NaCl.

FEB 2 — 3 l. day old culture of I coned. as before to 200 cc., pptd. w. 250 cc. alc. FEB 4 — Dark supernatant —, sucked off, ppt. centfgd., giving 3-layer sepn., of which top & bottom —. Middle layer taken up in H_2O, centfgd. Clear soln., 95 cc., pptd. w. 120 cc. alc. FEB 5 — Supernatant, —, sucked off, ppt. centfgd., drained as well as possible, diss. in 40 cc. H_2O, centfgd., clear soln. pptd. w. 80 cc. alc. FEB 6 — Supernatant —, sucked off, ppt. centfgd. FEB 7 — Ppt. diss. in H_2O, acidif. w. HOAc, centfgd. Clear soln., 25 cc., pptd. w. 25 cc. alc. After several hrs. centfgd. FEB 8 — Top & bottom layers —, discarded. Ppt. diss. in H_2O, centfgd., pptd. w. $(NH_4)_2SO_4$. FEB 11 — Supernatant poured off, ppt. redis. in H_2O, centfgd., not quite clear soln. (ca 10 cc.) again pptd. w. $(NH_4)_2SO_4$. Portion saved.

(NH4)2SO4 1

Am Sulf 2

MAR 12 — Concentrate from 6 l., from day before, at vol. of 320 cc., pptd. w. 400 cc. alc. MAR 13 — Supernatant —, drawn off, ppt. centfgd. Top layer —, bottom +, discarded, middle diss. in ca. 90 cc. H_2O centfgd., & at vol. of 100 cc., pptd. w. 150 cc. alc. MAR 14 — Supernatant —, discarded ppt centfgd., diss. in H_2O, centfgd., & at vol. of 40 cc., pptd. w. 50 cc. alc. MAR 15 — Supernatant —, discarded, ppt. centfgd. Redis. in H_2O, centfgd., acidif. w. HOAc, centfgd., soln. from previous sepn. added, & both pptd. w. = vol. alc. Supernatant —, ppt. centfgd., diss. in H_2O, centfgd., soln. acidif. w. HOAc, again pptd. w. = vol. alc. MAR 21 — Ppt. taken up in H_2O, centfgd. at vol. 15 cc. from considerable insol. portion, soln. made alk. w. N NaOH, pptd. w. 2 vols. alc.

S. S. S. 32

MAR 22 — Supernatant —, discarded, ppt. diss. in H_2O, centfgd, acidif. w. HCl + diald. in parchment. MAR 24 — Cl^- free. Concd. dry on H_2O bath, taken up in hot H_2O, centfgd. from opious residue. Soln. concd. dry in weighed dish.

Wt. dish + subst	28.652
" "	28.555
" subst	.097

MAR 25 - Diss. in saline, centfgd. from trace of ppt., made up to 5 cc.
Activity evident only as far out as 1: 5,000.
Biuret — Very faint if at all (opalescence of soln. makes decision difficult).

Soluble Specific Substance 34 — Type I

SS 34

Conc.	Tag Nos.		No. Flasks		Ppt'd	Trans. To Stock
3/17/24	I/191/19	III - 8	14		3/18	3/20
3/18	"	"	12		3/20	3/24
3/19	"	"	14		3/20	3/24
3/20	I/191/19 a 20	III - 8, 10	3 & 11		3/21	3/25
3/21	I/191/20 / 188/36	III - 10, 14	1 & 9	6H	3/22	3/25
3/24	I/188/36 & 37	III - 14, 15	1 & 13	34 / 10	3/25	2/26
3/25	I/188/36 a 37	III 14, 15	2 & 9		3/26	3/28
3/26	I/188/37 a 38	III 15, 17	10 & 4		3/27	3/28

Total 103 flasks

154.5 liters

MAR 28 1924 — Stock bottle removed from ice-box, about half poured out, rest rinsed out w. little H_2O & warmed on H_2O bath until salts diss. Run through Sharples with large nozzle. Soln., 2 at vol. of 2200 cc., in 2 parts, ppt'd. w. 2600 cc. alc., MAR 29 — Still +, so 400 cc. more alc. added MAR 31 — Supernatant, —, sucked off, ppt. centfgd. Very small middle layer, with top & bottom layers —. These discarded. Ppt. taken up in hot H_2O & centfgd. repeatedly at high speed. APR 1 — Ppt. washed w. H_2O. At vol. of 450 cc., ppt'd. w. 3 700 cc. alc. APR 2 — Supernatant, —, sucked off, ppt. centfgd. Diss. in hot H_2O, centfgd. repeatedly, ppt. washed 2 x w. H_2O. APR 3 — Soln. 4 at vol. of 275 cc., + 5 g. NaOAc, ppt'd. w. 400 cc. alc. Supernatant, —, sucked off, ppt. centfgd. APR 4 — Ppt. diss. in hot H_2O, centfgd. Soln. rinsed into centfg. bottle, & at vol. of 250 cc., 2 g. HOAc added. Ppt. centfgd. off., washed w. H_2O. At vol. of 275 cc., ppt treated w. 5 g. 5 NaOAc & 400 cc. alc. added. APR 5 — Supernatant, —, decanded, ppt. redis. in H_2O, HOAc added, & ppt. centfgd. (& washed w. H_2O.) 3 g. NaOAc 6 in little H_2O added. At vol. of 200 cc., ppt'd. w 250 cc. alc. APR 7 — Supernatant —, discarded, ppt. centfgd. off, diss. in H_2O, centfgd. 7 Clear soln. + 5 g. NaOAc + 50 cc. 10% NaOH, at vol. of 200 cc., ppt'd. w. 250 cc. alc. Supernatant —. APR 8 — Drawn off, ppt. centfgd. Amt. very small, perhaps due to too strong alkali. Redis. in cold H_2O, acidif slightly w. HOAc, & at vol. of 50 cc., 30 cc. alc. added, soln. remaining slightly opalescent. Left in ice-box in stoppered flask. 8 SEP 26 — Warmed to room temp. 3 cc. alc. added to opalescent soln. causing ppt. SEP 27 — Centfgd. Supernatant —. Ppt. washed w. a little slightly stronger alc. SEP 29 — Diss. in H_2O, opalescent soln. concd. dry. Taken up in hot H_2O, centfgd., considerable amt. ppt. washed w. H_2O. Soln. & washings

again concd. dry on H_2O bath. No insol. matter when again taken up in H_2O. Filtered through Berkefeld W, but still opalescent. Filt. & washings concd. ~~to small bulk~~ dry on H_2O bath. Taken up in hot H_2O, centfgd.

OCT 3 – Aq. sol. of ca. 10 cc., 2 cc. 1:1 HCl added, subst. ppt'd. w. 20 cc. alc. Centfgd, & supernatant, –, discarded. Ppt. taken up in ca 10 cc. H_2O & centfgd., considerable insol. matter remaining. Soln. poured into Collodion dial. bag & dialyzed, 1 cc. 1:1 HCl added, & dialyzed agst. distd. H_2O.

Insol. material diss. w. more H_2O, but soln. only very weakly +.

OCT 7 – After dialysis main soln. only ±. Did active material dialyze, or was it hydrolyzed by HCl?

Soln. discarded.

SS 35

Rec'd	Tag Nos.	Tag Date	No. of Fl.		Precip.	Stocked
3/31/24	I/188-192/38-3	III-17, 22	1 + 13	1	H/1	H/2
H/1	I/192/3	III-24	14	2	H/2	H/3
H/2	I/192/3,4	III-(22-24),(24,26)	2,5,2,5	3	H/3	H/4
H/3	I/192/3,4	III-22,(25-29)	10,2,2	4	H/4	H/5
H/7	I/192/3	III-28	11	12	H/8	H/9
H/8	I/192/4,5	III-(24,25),28	1,1,12	5	H/9	H/10
H/9	I/192/3,4	III-28,29	8,6	6	H/10	H/11
H/10	I/192/4	III-29	11	13	H/11	H/14
H/11	I/192/4,5	III-29,31	6,5	14	H/14	H/15
H/14	I/192/4,5	III-29,31, IV-5	1,1,9	15	H/15	H/16
H/15	I/192/5,6	IV-5, 7	3,11	7	H/16	H/17
H/16	I/192/5,6	IV-5, 7	9,5	8	H/17	H/18
H/17	I/192/4,5,6,8	III-29, IV-5,7,11	1,1,3,9	9	H/18	H/19
H/21	I/192/8	IV-11	14	10	H/22	H/23
H/22	I/192/6,8,9	IV-7,11,14	1,2,11	11	H/23	H/24
H/23	I/192/8,9	IV-11,14	1,9	16	H/24	H/25

11 × 14 = 154
54
208

Notes by Francis Catesi

Total Flasks	208
Total Liters	312 ✓

— Purification —

H/26 There were about 2800 cc or 2900 cc. of SS. in bottle.
Poured off top (clear solution) into beaker.
Rinsed crystals into another beaker adding about 450 cc. of warm water. When crystals were dissolved whole of solution was put thru Sharples (medium nozzle).
Rinsing water used, about 800 cc.
Total volume of solution — 4050 cc
" " " alcohol — 4050 × 1.4 = 5670 cc

2

H/28 Tested supernatant. Found positive. Added 500 cc alcohol.
Made test — negative.

Purification of NN35 (continued)

Filled nearly 6 (250 cc) centrifuge bottles with precipitated NN.
Centrifuged. Good separation. Dissolved middle layer.
Centrifuged. Poured-off clear solution into a four liter
3 separatory funnel. Washed. Centrifuged. second washing.
Total volume of solution 950 cc.
" " " alcohol 1400 cc.

4/29 Tested supernatant. → negative. Centfgd. Good separation. Dissolved middle layer. ~~Centrifuged~~. Washed precipitate twice ~~second time~~. Centrifuged.
Total volume of S.S. 560 cc. Added 10 gms acetate.
4 " " " alcohol 800 cc

4/30 Tested supernatant → negative. Centrifuged. Good separation. Dissolved in running and hot water. Washed precipitate. Added acetic acid. Centrifuged. Poured-off
HOAc 5 clear solution into two liter funnel. Washed precipitate.
Total volume of S.S. 550 cc. Added 10 gms of acetate.
" " " alcohol 800 cc

5/1 Tested supernatant — negative. Centrifuged. Dissolved.
Added 5 cc of acetic acid. Centrifuged. Washed precipitate.
6 Total volume of S.S 425 cc. Added 6 gms acetate with water.
" volume " alcohol 530 cc

5/2 Tested supernatant → negative. Centrifuged precipitate.
Dissolved in hot water. Centrifuged. Washed precipitate.
Volume of S.S. 300 cc
Added Alcohol 75 cc

SEP 29 — Rather heavy ppt. Supernatant, weakly +, decanted, ppt. centfgd. in cold. 125 cc. alc. added to supernatant without giving immed. ppt.

OCT 1 — Combined w. 35 A.

Ppt. combined w. centfgd. ppt. of 35 A, supernatant combined w. that from 35 A, concd. on H_2O bath (Soln. B). At vol. of ca. 200 cc. 125 cc. alc. added. ~~Ppt~~ Ppt. Centfgd. ~~ppt~~ OCT 2 — Supernatant —, poured off, ppt. dis. in ca 100 cc. H_2O, ppt'd. w. ca 40 cc. alc. Stood in cold. Supernatant —, poured off. Ppt. dis. in H_2O, soln. added to that of main 35 ppt. & these combined w. soln. of 35 A. Let stand in ice box.

On continuation see p. 4.

SS 35A

	Tag Nos.	Tag Date	No. of Fla.	Precip.	Stocked
4/24/24	I/192/9,10	IV-14,15	4, 5	4/25	4/26
4/28	1/192/7	IV-19	14	4/29	4/30
4/29	1/192/7	IV-19	14	4/30	5/1
4/30	1/192/7,8	IV-19,21	6, 8	5/1	5/2
5/1	1/192/7,8	IV-19,21	1, 9	5/2	5/3
5/2	1/192/7,8	IV-19,21	1, 8	5/3	5/5
5/6	1/188/37	IV-29	14	5/7	5/8
5/7	1/188/37	IV-29	14	5/8	5/9
5/12	1/188/35	V-3	14	5/13	5/14
5/13	1/188/35,37	V-3, IV-29	3, 11	5/14	5/15
5/14	1/188/35	V-3	14	5/15	5/16
5/19	1/193/23	V-10	14	5/20	5/21
5/20	1/193/23	V-10	14 (180)	5/21	5/22
5/21	1/193/23	V-10	12	5/22	5/23
5/26	1/193/22	V-19	12	5/27	5/28
5/27	1/193/22,23	V-19, 20	8, 6	5/28	5/29
5/28	1/193/23	V-19, 20	10, 4	5/29	5/31

Unusual Behavior. Put aside. ↓

Set stand in ice-box, gave supernatant. Ppt'd layer dis. w. warm H_2O, ppt'd. w. alc., middle layer dis., centfgd., added to 37

Ppt'd as in 35, but middle layers taken up in H_2O, centfgd., ppt. washed, solns. & washings transf. to stock bottle.

Notes by Francis Cataldi

T. FLASK 220 − 12

T. LITERS 330 − 18

312 liters

6/2 There were about 4 liters of clear, centrifuged

S.S. This was poured into calibrated jar and precipitated with 4000 × 1.4 = 5600 cc. of Alcohol.

2

6/3 Tested supernatant. Found positive. Add 500 cc of Alcohol. Tested — negative.

Centrifuged (6, 250 cc bottles). Good separation. Redissolved middle layer. Centrifuged. Poured off clear solution into 4 L. funnel. Centrifuged second washing.

Purification S.S. 35A (continued)

Total volume S.S. 900 cc
3 " " alcohol 1400 cc

6/4 Tested supernatant — negative. Centrifuged. Dissolved middle layer. Washed precipitate ~~second time~~ twice. Centrifuged. Washed precipitate
4 third time. Centrifuged.
Total volume of S.S. 525 cc + 10 gms acetate.
" " " alc. 800 cc

6/5 Test supernatant — positive. Added 100 cc alc. Tested — negative. Centrifuged. Dissolved middle layer. Washed precipitate. Added acetic acid as per direction. Centrifuged, until clear. Washed precipitate with few drops of acetic acid. (H_2O cont.)
5 Total volume S.S. 550 cc + 10 gms of acetate
" " alc. 800 cc.

6/6 Tested supernatant — negative. Centrifuged. Dissolved middle layer. Added 5 cc acetic acid.
6 Centrifuged. Washed precipitate
Volume of S.S. 400 cc + 6 cc acetate and water.
" " alc. 500 cc

6/9 Tested Supernatant — negative. Centrifuged Dissolved in hot water. Centrifuged.
No. precipitate
Total volume S.S. 300 cc
" " alc. 75 cc

OCT 1 — Heavy ppt., as in 35, Supernatant, weakly +, decanted, concd. to sm. bulk w. that from 35. Ppt. centfgd. in cold OCT 2 Disp. in H_2O, added to 35.

OCT 3 — Combined solns. centfgd. from considerable insol. matter in cold centfg. Ppt. washed w. H_2O. At vol. of 230 cc. contg. 10 cc. 10% NaOH
7 & 5 g. NaOAc, ppt'd. w. alc. 80 cc. & 1" slight ppt. Centfgd. Ppt., almost —, added to 36, ~~fol~~ supernatant ppt'd. w. 220 cc. more alc. Supernatant now ±.

OCT 4 — Sucked off, ppt. centfgd., diss'd. in cold H_2O, acidified w. HCl & centfgd. Insol. portion redissolved when taken up in H_2O, soln. ++++

S. S. 35

so combined with rest. 10cc. 10% NaOH added, & at vol. of 150cc., ppt'd. w. & 150cc. alc. Soln ++, so 100 cc. more alc. added. OCT 7 - Supernatant -, so sucked off, ppt. centfgd., diss. in H_2O. OCT 8 - Soln. still slightly colored, 9 so at vol. of 90 cc. again ppt'd. w. 100 cc. alc. Set stand in ice-box. OCT 9 - Supernatant -, sucked off, ppt. centfgd. in cold, diss. in H_2O. If too little H_2O used much remained insol. OCT 10 - Soln. filtered through 5 in Berkefeld N candle, then through W, but still somewhat opalescent. OCT 11 - Concd. to 75 cc. on H_2O bath, but on acidif. w. HCl gave no ppt. Concd. to 55 cc. OCT 20 - Strongly opalescent soln. shaken w. "Norite," but still remained milky. Little $Pb(OAc)_2$ soln. is added to dild. mixt. & H_2S passed in. OCT 21 - Norite filtered off, black soln. concd. dry on H_2O bath. OCT 22 - Taken up in hot H_2O, centfgd, decanted from sm. amt. ppt., again concd. dry. Taken up in hot H_2O, centfgd. OCT 23 - Process repeated. OCT 24 - Process repeated. OCT 25 - At vol. of 200 cc., soln. + 5 g. NaOAc treated w. 130 cc. alc. no noticeable ppt. OCT 27 - As sm. amt. black material had ppt'd., this centfgd. off, washing in w. little alc. of same concn. Both ppt. & supernatant ++, so soln. ppt'd - w. 100 cc more alc, taking down all of Pb, too. Supernatant -, decanted, ppts. redis. in H_2O. OCT 29 - At vol. of 200 cc, treated in cold w. 100 cc. HCl & evacuated to remove H_2S. Color now pale brown. Partially neut. w. NaOH & ppt'd. w. alc. Set stand in ice-box. OCT 30 - Ppt. centfgd. off. (B). Supernatant + Et_2O, sepd. into 2 layers. Upper, -, discarded. Lower, neut. w. NaOH, gave ppt. Mixt. centfgd. Ppt. contained all active material. Dis. in H_2O & added to aqs. soln. of B. Little $(NH_4)_2SO_4$ added, ppting. $PbSO_4$. Centfgd. Soln. ppt'd. w. $(NH_4)_2SO_4$, centfgd. Supernatant preserved, ppt. diss. in ca. 50 cc H_2O, again satd. w. $(NH_4)_2SO_4$, centfgd. Supernatant - ppt. diss. in 50 cc. H_2O, centfgd. Soln. only ±, so discarded. Main supernatant centfgd., clear soln. dialyzed agst. running H_2O. NOV 19 - Concd. on H_2O bath, centfgd, clear soln, at vol. of 72 cc, treated w. 2.1 cc. HOAc, to make concn. of N/2. Slight turbidity, but no unmed. ppt. Set stand in ice-box. NOV 20 - Small amt. ppt. centfgd. off. Supernatant very weakly +. Ppt. diss. w. few drops N NaOH & added to corresponding soln. of (37)

SS 36

	Tag Nos.	Tag dates	No flasks	Grew	Stock
5/29/24	1/193/23	V - 19, 20	9, 1	6/3	6/4
6/5	1/188, 193/37, 22, 27	V - 19, 20, 27	1, 1, 12	6/6	6/7
6/6	1/188/37	V - 27	12	6/7	6/9
6/9	1/188/37	V - 27	12	6/10	6/11
6/10	1/193/23	VJ - 3	14	6/11	6/12
6/11	1/191/23	VI - 3	14	6/12	6/13
6/12	1/191/23, 24	VI - 3, 6	1, 13	6/13	6/14
6/13	1/191/23, 24, 25	VI - 3, 6, 7	1, 12, 1	6/14	6/16
6/16	1/191/24, 25	VI - 6, 7	13, 1	6/17	6/18
6/17	1/191/25	VI - 7	13	6/18	6/19
6/23	1/193/8	VI - 14	14	6/24	6/25
6/24	1/193/8	VI - 14	14	6/25	6/26
6/25	1/193/8, 9	VI - 14, 16	2, 12	6/26	6/27
6/26	1/193/9	VI - 16	8	6/27	6/28
7/1	1/188/37	VI - 23	14	7/2	7/3
7/2	1/188/37	VI - 23	14	7/3	7/7
7/8	1/188/38	VI - 27	11	7/9	7/10

Total flasks 220
Total liters 330

Treated as in 35A.

7/11 There were about 4 liters of clear centrifuged S.S. ydr, and precipitated with: This was poured into calibrated

4100 x 1.4 = 5740 cc. AH Cohn

7/13 Test supernatant — negative. Centrifuged (6, 250 cc) Good separation. Dissolved middle layer. Centrifuged. Poured-off clear solution into

Purification SS 36 (continued)

Ppt. washed x x.
funnel. ~~Centrifuged second washing.~~
3 Total volume of SS 900 cc.
" " " alcohol 1400 cc

7/14 Tested supernatant – negative. Centrifuged. Dissolved middle layer. Washed precipitate. Centrifuged. Washed precipitate again.
4 Total volume of SS 550cc + 10 gms acetate.
" " " alcohol 800 cc.

7/15 Tested supernatant – negative. Centrifuged. Dissolved middle layer. Washed precipitate. Added acetic acid as per directions. Centrifuged until clear. Washed precipitate
5 with a little water containing a few drops of acetic acid. Centrifuged.
Total volume of SS 550 cc + 10 gms acetate.
" " " alcohol 800 cc.

7/16 Tested supernatant – negative. Centrifuged
6 Dissolved precipitate in hot water. Added 5 cc acetic acid. Centrifuged. Washed precipitate.
Total volume of SS 1400 cc + 6 gms acetate.
" " " alcohol 500 cc.

7/17 Tested supernatant – negative. Centrifuged. Dissolved in hot water. Centrifuged. Washed precipitate.
7 Volume of SS 300 cc.
" " alcohol 75 cc.
Labelled, & stand in ice-box.

S. S. S. 36

OCT 20 — Supernatant, slowly ±, sucked off, ppt. centfgd. in cold.
OCT 21 — Diss. in H_2O, centfgd., washing ppt. thoroughly. Soln. let stand in ice-box. OCT 23 — At vol. of 250 cc. centfgd. in cold centfg. until clear. OCT 24 — As washings of ppt. were strongly opalescent,
8 washings added to (37). Soln. +5g NaOAc pptd. w. 70 cc. alc., & let stand in ice-box. OCT 25 — Supernatant —, mixt. centfgd. in cold centfg.
OCT 27 — Ppt. taken up in ca. 200 cc. H_2O, 10 cc. 10% NaOH added, centfgd. in cold centfg. Ppt., rediss. in H_2O, highly opalescent, so added to (37). Main soln., at vol. of 225 cc., pptd. w. 75 cc. alc., set in ice-box. Supernatant, centfgd., still +++, so 50 cc. more alc. added. OCT 28 — 200 cc. more alc. added. Centfgd. Supernatant, +, pptd. active material when neutr. This centfgd. off, & aq. soln., highly colored, added to (37). Main ppt. diss. in H_2O, centfgd., ppt. dissg. in more H_2O, but partially repptg. when added to main soln. 3g. NaOAc & 10 cc. N NaOH added. At vol. of 120 cc.
9 pptd. w. 200 cc. alc. OCT 30 — Supernatant, —, siphoned off, ppt. centfgd. in cold, diss. in H_2O. Turbidity centfgd. off, proved to be liquid layer. Main soln. (A). Lower layer (B). Sepd. as cleanly as possible. With a little more H_2O B formed homogeneous aq. soln., slightly opalescent.

A

Satd. w. $(NH_4)_2SO_4$, giving slight ppt. OCT 31 — Repeatedly centfgd. Almost clear supernatant poured off, ~~ppt. taken up in~~ & soln. from
$(NH_4)_2SO_4$ B ppt. added. Ppt. taken up in just enough H_2O not to diss. all $(NH_4)_2SO_4$ & again centfgd., adding soln. to main one. Ppt. taken up in H_2O, added to suspension of repptd B material. Main soln. filtered in attempts to remove last traces of ppt., but these passed through. NOV 4 — Soln. again centfgd. in cold at high speed, removing practically all of ppt. Soln. diald. agst. running H_2O ~~over night~~ for 2 days.
NOV 7 — Concd. in vac. Does not ppt. on addn. of HCl. At vol. of ca. 200 cc.
10 pptd. w. 100 cc. alc. & let stand in ice-box. NOV 8 — Supernatant —, so centfgd., most of $(NH_4)_2SO_4$ remaining in soln. Ppt. washed 1" w. 55 cc. 50% alc. NOV 10 — Centfgd. NOV 17 — Supernatant —, discarded, still much $SO_4^=$. Ppt. washed w. 100 cc. 75% alc., centfgd. in cold. NOV 18 — Supernatant — contains $SO_4^=$. Ppt. macerated in cold N/2 HOAc, part apparently dissg. 100 cc. used. Centfgd. in cold. Supernatant —, red color w I. Ppt. washed w. 100 cc. N/2 HOAc, let stand ½ hr., centfgd. as before. Still faint color w I. Diss. w. aid of 25 cc. N HCl, 25 cc. H_2O, 4 g. NaOAc added, but very little ppt. Centfgd. in cold. Supernatant ++++. More came down on standing over night, but supernatant still +++

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NOV 21 — Supernatant (C) transf. to sep. funnel, ppt. (A) washed w. 50 cc. $\frac{N}{2}$ HOAc + centfgd.
NOV 22 — (C) at vol. of 125 cc., pptd. w. 50 cc. alc. Supernatant over A, —, also free from glycogen by ~~[illegible]~~ test, discarded. Ppt. from (C) (supernatant —) + (A) diss. in H_2O + xs. ~~NaOH, & at vol. of 225 cc., pptd. w. xs. alc. Supernatant (D) still active, neutr., & cond. on H_2O bath. Ppt. centfgd. off, diss. in H_2O.~~ N HCl, dial'd. agst. running H_2O, ppt. gradually forming. NOV 28 — Transf. to distd. H_2O. NOV 29 — H_2O ch. DEC 1 — Still sm. amt. Cl^- in dialysate. H_2O ch. DEC 2 — H_2O ch. DEC 3 — Cl^- negative inside & out. Contents of bag rinsed into Buchner in ice-box & sucked off. Filtrate now called (C). DEC 4 — Washed w. little alc., then w. acetone, warmed to ca. 40°, dried in vac. Yield: 1.5 g.
Portion ground up, dried to const. wt. in vac. at 100°

Tests on 1:200 Soln.

Biuret: Negative
I-KI: No color
Ninhydrin: No color; reddish brown after hydrolysis (1:800), soln. cont. NH_3
$KMnO_4$: No immed. redn.
HCHO: Specificity not lost on standing over night w. 1/5 vol. 40% HCHO soln.

DEC 13 — Test to det. whether NH_2-N is due to impurity or to S.S.S. itself. 0.1 cc. ea. 36, 25A, 31 (I, II, III) added to 0.3 cc. 30% $NaNO_2$ soln. 0.1 cc. HOAc added to ea. Shaken by hand 10 min. Dil. w. H_2O, neutr. w. NaOH, all made up to ca. 5 cc. to give approx. 1:10,000 solns. Tests agst Sp. Sera:

Type I	Type II	Type III
0	+++	+++

Appears to show that NH_2 N an essential part of SS I mol.

Action of N/2 NaOH

DEC 24 — 0.1 cc. soln. + 0.1 cc. N NaOH htd. in steam bath. During several hrs. in open tube soln. evapd. and discolored, but still markedly sp.

[Specificity of original soln. 1:6,000,000.

JAN 2 1925 — Action of N NaOH. 1.5 cc. soln. + 3.5 cc. H_2O dil'd. to 10 w. 2N NaOH. Immersed in b. H_2O bath & slow stream air, washed w. Na-lime & hot dil. H_2SO_4 passed through & into 2.1 cc. N/50 HCl. Cc. N/100 NaOH (×1.014) 13.90 4.20
Htd 5 hrs. $N = \frac{1.26 \times .14}{7.5} = 2.4\%$ Soln. still weakly + 11.00 2.94
Attempted to continue next day, but lost. 2.90 1.26 .82

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JAN 15 – Test w. HNO_2 repeated to meet objection that loss of specificity might have been due to any HNO_3 present.

① 0.1 cc. S.S.36 (1:200), 0.2 cc. H_2O, 0.1 cc. HOAc, 0.1 cc. colorless conc'd HNO_3.

② 0.1 cc. " " " " 0.3 cc. 30% $NaNO_2$, 0.1 cc. HOAc.

① & ② shaken 10 min, then dil'd. w. H_2O, neutr. w. N NaOH, dil'd. to ca 5 cc. ea. Tested w. clear 2:3 Type I serum.

① +++, ② –.

Test ② repeated on 0.3 cc. soln. & dil'd. only w. the N NaOH used for neutr. With Fehlings soln: Redn: +++

Blank w. $NaNO_2$ & NaOAc : " : –

" " untreated S.S.36 : : ±

Removal of N by HNO_2 accompanied by appearance of redg. sugar shows poly-glucosamine or analog.

G. Karrer et al. Helv. Chim. acta (1924).

$UO_2(NO_3)_2$ – Gelatinous ppt.

S.S.S. 36

DEC 4 — Wt. bot (12) + subst 8.0755 8.0751 8.0742 Htg. contd. in $CHCl_3$
vapor, as color gradually darkened 8.0750
7.9929
.0821

8.0000
71
7.9929

Drop phthl. added, titr. w. 14/14 NaOH (1.006) 3.52 cc.
" HCl (.992) 1.72

Soln. made up to 16.42 cc.

Broad zone in which pink color fades almost imperceptibly to faint yellow — Endpt.

0 = +0.01 .01 ~~3.08 2.96 2.88~~
3.00 2.99 2.98

$[\alpha]_D = +300°$

Ash-free $[\alpha]_D = +304.5°$

DEC 6 MICROKJELDAHLS

	I	HCl as N/100	II	HCl as N/100	Bl.	HCl as N/100
1 cc. samples.						
cc. N/50 HCl (x 1.001)	2.00		2.10		2.00	
" N/100 NaOH (x 0.997)	9.94		12.00		15.51	
	8.05	4.00	9.97	4.20	12.00	4.00
	1.89	1.88	2.03	2.02	3.51	3.50
		2.12		2.18		.50

In 1 cc. sample 0.223 mg. N
" 20 cc. (100 mg.) 4.46 mg. N

Mean: 2.15
Bl. .56
1.59

Goebel Bl. .62
Mean: .56

$N = 4.5\%$

Ash-free $N = 4.6\%$

DEC 8 ASH

Wt. bot. + subst 7.9933
" " 7.9463
.0470

Wt. dish + ash 23.4035
" " 23.4018
Wt. ash as $CaSO_4$ .0017
" " " CaO .0007

Burned w H_2SO_4. Addn. of 30% H_2O_2 nec. before all C oxd.

% Ash 1.5%

HYDROLYSIS

3 cc. dil. to 6, 6 cc. 2N HCl added, b. 4 hrs.
1.5 cc. samples cc. $Na_2S_2O_3$

I	II	Mean:	Bl.
17.75	17.63	17.70	19.8
			17.7
			2.1 cc.

2.1 x .981 x .318 = 0.655 mg. Cu = 0.41 mg. glucose
1.875 mg. in 1.5 cc. Red. Sug. 21.9%

Soln. b. 1 hr. longer
2 cc. sample cc. $Na_2S_2O_3$ 15.45 Bl. 19.8
4.35 x .981 x .318 = 1.36 mg. Cu = 0.68 mg. glucose 15.45
2.5 mg. in 2 cc. Red. Sug. 27.2% 4.35

Soln. b. 1 hr. longer
2 cc. sample cc. $Na_2S_2O_3$ 14.95 19.8
14.95
4.85

DEC 9 — New HYDROL. 6 hrs. 2 cc. cc. $Na_2S_2O_3$ 14.55 19.8
5.25 x .981 x .318 = 1.64 mg. Cu = 0.79 mg glu. 31.6% 14.55
1 hr. longer cc. $Na_2S_2O_3$ 14.42 5.25

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Amino N Detns.

DEC 10

Hydrolysis soln. (2nd) 2 cc.

t	Barom	Rdg.	Corr.	− Bl.
22	767.3	0.100	0.99	checking w. blank on 29 B
		0.323	.322	$\frac{.223 \times .5688 \times 100}{2}$ = 6.3 mg. NH_2 N per 100 cc. ✗ Soln. not counted.
22.5		0.250 →	.249	$\frac{.150 \times .5670 \times 100}{2}$ = 4.3 " " " " Mean: 4.5
23°		0.265	.264	$\frac{.165 \times .5663 \times 100}{2}$ = 4.7 mg " " " "
				9 in 100 cc. : 125 mg. N = 3.6% Hydr.

Unhydrol. Soln. 1 cc. washed in w. 1 cc. H_2O.

22.5 0.310 .309 $\frac{.209 \times 5670 \times 100}{1}$ = 11.84 mg NH_2N per 100 cc.

9 in 100 cc. 500 mg. N = 2.4% Unhydr.

Ash-free Amino-N
2.4% before hydrol.
3.7% after "

DEC 11

HYDROLYSIS

2 cc. dil. & 4, 4 cc. 2N HCl added, & 7 hrs.
Dil. & 10 cc. 5 cc. used in ea. detn.

AMMONIA N Detns.

cc. N/50 HCl (x1.001)	Bl I	Bl I	Bl II	Bl II	Bl. I		Bl. II	
" N/100 NaOH (x0.997)	2.00		2.00		2.00		2.00	
	12.09		15.98		11.76		15.50	
	8.00		12.09		8.01		11.80	
	4.09		3.89		3.75		3.70	
Aq N/100 HCl		4.20		4.00		4.00		4.00
" " NaOH		4.08		3.88		3.74		3.69
		.12		.12		.26		.31

9 in ea. sample 0.024 mg. N as NH_3
5 mg. subst.

Mean Bl. 0.29 − .12 = .17

After Hydrol. N = 0.5%

JAN 13 1925 — C & H by Dr. Elock.

0.1020 g. gave 0.0524 g. H_2O + 0.1594 g. CO_2.

Ash-free subst. 0.1005 g.

Ash-free C = 43.26% H = 5.83%

432.	63548	64884	43573
60.48	00626	71933	20249
480.	62922	99783	99783
42.03	78161	76600	63605
1017.51	00626		
	77535		
	62356		
	00626		
	61730		

66276 / 14644 / 51632

wt. 53.70 / 46.30

C 43.26 ÷ 12 = 3.605
H 5.83 ÷ 1.008 = 5.79
N 4.6 ÷ 14.01 = .328
O 46.3 ÷ 16 = 2.80

$C_{36}H_{58}O_{29}N_3$
$C_{36}H_{57}O_{30}N_3$

S. S. S. 36

JAN 5 1925

Wt. bot(3) + subst dried const @ t.b. $CHCl_3$ 7.6794 7.5521
7.5519 7.4020
.1275 .1501

Diss. by ~~adding~~ suspg. in H_2O, adding N HNO_3 in xs., neutr. to phthalphal. w. NaOH. Made up to 30 cc. w. H_2O. Centfgd. from traces dirt.

JAN 7

Acid Equivalent (Silver Titration).

2 cc. sample. 5 cc. $AgNO_3$ soln. N/10 x 1.011. Ppt. centfgd. off, 2 cc. taken for titrn.

	I	II
cc. N/50 KCNS (x 1.03)	6.45	6.45
$\frac{2}{7}$ x 25 x 1.011 = cc. N/50 $AgNO_3$ for sample		= 7.22
cc. N/50 KCNS		6.64
		.58 cc. N/50 $AgNO_3$ used up.

2.86 0000 by ~~10~~ mg. subst. = 0.0116 cc. N

~~28.6~~ mg. = 116 cc. N $AgNO_3$

~~[illegible]~~ = Ag. Equiv. = ~~[illegible]~~ 247

Acid Equiv
251
Ash + sec

JAN 7

Hydrolysis in 2N NaOH

2 cc. dil'd. w. 8 cc. 10% NaOH, immersed in b. H_2O bath, using precautions in preceding expt. w. N NaOH. NH_3 passed into 2 cc. N/50 HCl

	After 3 hrs.	N/100	After 2 hrs. longer		N/100
N/50 HCl (x 1.001)	2.12	4.24		2.00	4.00
N/100 NaOH (x 1.014)	14.27		15.63	~~12.00~~	
	12.00	2.30	12.00		3.68
	2.27		3.63		.32
		1.94			
		~~Pl. .44~~			
		~~1.50~~			
NH_3 N		2.7%			.448
Total		.4			
		3.1%			

JAN 8 – Soln., neutr., gave heavy ppt. of H_2SiO_3. With this was a small amt. of subst., insol. in neutr. soln., sol. in acid. Supernatant from H_2SiO_3 very weakly +.

Silica was in such large amt. that concn. of NaOH must have been decidedly less at end of expt. Old, scratched Pyrex tube used.

S.S.S. 36

Alkaline Hydrolysis. Repetition w. 2 N NaOH in new Pyrex tube.

JAN 12 — After 2 hrs

		N/100	4hrs.	N/100	6 hrs.	N/100
cc. N/50 HCl (x1.001)	2.00	4.00	2.00	4.00	2.00	4.00
" N/100 NaOH (x1.014)	10.21		13.58		14.71	
	8.00	2.24	10.20	3.43	11.01	3.75
	2.21	1.76	3.38	.57	3.70	.25
NH_3 N		2.5% .09/1.69		0.8% .08/49		0.4% .08/.18
		.8%				
		4%				
Total		3.7%				

Corr. for Bl 2.4%
.7
.3
3.3%

JAN 13 — Ht g. cont'd.

	After 8½ hrs	11½ hrs	Blank		Blank	
cc. N/50 HCl (x1.001)			2.00	4.00	2.00	4.00
" N/100 NaOH (x1.014)	12.75	11.95	15.90		12.03	
?	8.00	8.10	12.05	3.90	8.15	3.93
		3.85	3.85	.10	3.88	.07
				Mean 0.08		

Test for Phosphorous

2 cc. soln. b. w. 2 cc. conc. HNO_3 + 1 cc. H_2SO_4 until white fumes appeared. Cooled, dild. w. H_2O, few drops conc. HNO_3 added, & $(NH_4)_2MoO_4$ added.
No ppt., even on standing over night.
Control w. reagent + $PO_4^{\equiv}$ gave good pptn.

No P

Test for Sulphur.

about 15 mg. dry substr. fused w. $NaNO_3$. Pungent odor given off. Fusion, taken up in H_2O, acidif. w. HCl, filtered. Remained clear when $BaCl_2$ soln. added.
Faint whirl from bottom on standing over night.

No S

S. S. S. 36

Hydrolysis w. HNO_2, followed by b. w. H_2SO_4

FEB 12 – 2 cc. soln. (Jan. 5), 4 cc. 30% $NaNO_2$ soln., 2 cc. HOAc well shaken, let stand 0.5 hrs., w. occasional shaking. HNO_2 aspirated out, after adding 10 cc. 2N H_2SO_4. Soln. made up to 20 cc.

OPTICAL ROTATION

$l = 2$ $\quad 0 = -0.03 \quad -0.03$ Rdgs. $+0.11 \quad +0.12 \quad +0.12$

$$[\alpha]_D = \frac{.15 \times 100}{2 \times .05} \times \frac{100}{98.5} = +152°$$

Soln. transf. to hydrolysis app. & B. 2 hrs. ~~Let stand in ice box.~~

After 2 hs boiling. $l = 2$ $\quad 0 = .01 \quad .01 \quad .02 \quad .02 \quad .02$
Rdg = $.08 \quad .08 \quad .08 \quad .08$

Sugar Analysis 3 c.c. of Soln used (or $\frac{3}{20} \times 10 = 1.5$ mg)

c.c. Thiosulphate	17.90	
Blank (20.05 – " x .318) =	.66 mg	Copper Equiv.
Glucose Equiv. =	.41 mg	
% Glucose	$\frac{.41 \times 100}{15} = 27.3\%$	

$[\alpha]_D = +102°$

B 2 hrs. longer, let stand over night in ice box.

$l = 2$ $\quad 0 = .01 \quad 0.00 \quad +0.02$ Rdg $+0.09 \quad .12 \quad .12 \quad .12$

$$[\alpha]_D = +102°$$

At vol. of 16 cc., concd. on H_2O bath to 4 cc., B. under ref. 2 hrs.

Bf. 20.05 / 16.75 $\quad$ Rdg. 16.75 $\quad$ 16.75 $\quad$ 1 cc. sample

$3.30 \times .957 \times .318 = 1.0$ mg. Cu $= 0.54$ mg. Cu

$\frac{.54}{2} =$ Redg. Sugars 27.0%.

S.S.S. 36
Acid Hydrolysis

JAN 7 1925 — 0.44 g. partly-dried substr., 0.16 g. in neutr. aq. soln., 0.6 g. in all, made up to 60 cc. w. H_2O, 60 cc. 2 N HCl added, b. 7 hrs. under rf. Set in ice-box JAN 9 — Shaken w. a little bone-black, filtered, washed w. N/2 HCl. Soln. still dark, still +++. 60 cc. more N HCl added & soln. b. 2 hrs. Cooled, let stand in ice-box. JAN 10 — Still trace of activity, so b. 2 hrs. longer. Cooled, let stand in ice-box. JAN 12 — Still +, so b. 2 hrs. longer. Still +, so 60 cc. more N HCl added, b. 2 ½ hrs. longer. Still ±. JAN 13 — Filtered from deposited C, & brown-yellow soln. Treated w. aqs., Et_2O-purif. HPW. 4.5 g. necessary for complete ppt'n. Let stand in ice-box. JAN 14 — Centfgd. Supernatant filtered. Xs $CuCl_2$ added, then neutr. w. NaOH & made just faintly acid w. HCl. Heavy, pale blue ppt. centfgd.-off. As supernatant had only slight redg. power & ppt. had carried down most of the sugars, ppt. ~~dissolv in xs. HCl~~ susp'd. in H_2O, diss. w. xs. 1:1 HCl, shaken out 2 x w. amylalc-Et_2O, then w. Et_2O, neutr. to Congo but not Litmus w. NaOH, & both this soln. & previous supernatant combined & satd. w. H_2S. Filtered. Filtrate intense blue, showing HPW had not been removed. Cncd. in vac. to 200 cc., filtered from S, 0.5 cc. Ph_1NHNH_2 added, neutr. to Congo w. NaOAc, let stand in ice-box. JAN 15 — Blue color gone. Yellow soln. filtered from traces tar, htd. on H_2O bath. After ½ hr. again filtered from traces tar. ~~On standing over~~ Htd - ca. ¼ hrs. longer, again filtered from traces tar, let stand over night. Small quantity brown material deposited, mainly amorphous, but with a few crystals present. Discarded.

S.S.S. 36

Acid Hydrolysis.

JAN 14 - A - HPW ppt. Washed w. N HCl centfgd. drop HPW, again centfgd. As ppt. showed redg. action, mixt. htd. w. dil. HCl on H_2O bath, part of ppt. melting. JAN 22 Soln. not redg, so discarded. Ppt. diss. in H_2O by adding N NaOH in sl. xs. Brown soln. ppt'd. w. xs. $BaCl_2$, giving sm. amt. brown ppt. of $BaHPW_2$(?) Yellow clr. soln. filtered & acidif. w. 1:1 HCl, giving gelatinous ppt. Centfgd. Ppt. gave weak glucuronic acid test w. naphthoresorcin + HCl. Filtrate from this ppt., after removal of Ba w. H_2SO_4 & SO_4 w. traces Pb OAc., gives slight ppt. on adding NH_3. ~~Ppt.~~ Centfgd. off. Redissd. in dil. HOAc, reppt'd w. sl. xs. NH_3, centfgd. Ppt., diss. in H_2SO_4 & filtered, does not give ~~neg~~ Legal-Curb Molisch test. Gives green underneath, brownish above.

S. S. S. 36 B

OCT 30 – Soln. B satd. w. $(NH_4)_2S$, giving relatively much ppt.
OCT 31 – Mixt. centfgd. Supernatant +, added to corresponding soln. of A. Ppt. rediss. in ca. 25 cc. H_2O, again pptd. w. $(NH_4)_2SO_4$. Centfgd. Supernatant, –, discarded. Ppt. suspd. in H_2O, soln. of corresponding material from A added, reppt'd. w. $(NH_4)_2SO_4$. Centfgd. NOV 1 – Supernatant discarded, ppt. diss. in ca. 30 cc. H_2O, centfgd., poured from slight ppt. into dialysis bag, dial'd. agst. running H_2O. NOV 6 – Soln. concd. to dryness, taken up in hot H_2O, filtered into redistd. acetone. NOV 7 – Filtered off, washed w. redistd. acetone, dried. Yield: 1.5 g.

Gives reddish color w. I, indicating glycogen or erythrodextrin.

S. S. S. 36 B

NOV 8 Rot. bot (3) + subst 7.3750 7.3747 Diss. in H_2O and made up to 15.08 cc.
7.2993
.0754

NOV 10 OPTICAL ROTATION
$\ell = 2$ $\alpha = +0.06$ .05 .05 Rdg. +2.01 1.96 1.94 1.96

$$[\alpha]_D = +190^\circ$$

Easily split reducing sugars by drop of saliva.