PROJECT REPORT FORM

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BACTERIAL CELLULOSE

SUMMARY

The original purpose of Project 1829 was to investigate the biosynthesis of cellulose at the cellular level. This was to be developed from two points of view, (1) the fundamental mechanism of biosynthesis of cell wall (cellulosic) material and (2) the possibilities of manipulating the synthesis of cell wall material for practical purposes.

After some preliminary work attempting to find an organism with relatively pure cellulose cell walls, the project developed into a study of the extracellular formation of cellulose by the bacterium Acetobacter sp. Various cultural conditions (time, temperature, pH, surface-to-volume ratio) were investigated relative to cellulose yield in a complex medium. The purpose was to optimize cultural conditions for high cellulose yield and maximum efficiency of incorporation of glucose into cellulose.

Additional experimental work was done on mass culturing of Acetobacter, the production and cellulose-synthesizing capability of cellulose-free cells, and bacterial cellulose as an additive to paper.

The project was closed in July, 1966. Although C-14 labeled glucose was used in earlier experiments, the acquisition of the Beckman liquid scintillation counter in 1967 made radioisotope studies more feasible. Consequently,

Project 1829 was re-opened in June, 1967, and several experiments were run designed to show the incorporation of D-glucose-UL-C-14 into cellulose with glucose and ethanol concentration in the growth medium as variables. Some experiments also included C-14 labeled ethanol, alanine, aspartic acid, and glutamic acid. Also, in cooperation with Paul Seib, the utilization of 2-fluoro-2-deoxy-D-glucose, D-mannose, 2-deoxy-D-glucose, and 3-deoxy-D-glucose by Acetobacter was investigated.

EXPERIMENTAL PROCEDURES AND RESULTS

RATE OF CELLULOSE FORMATION

The percent yield of cellulose with respect to time was determined in a medium containing 2.0% glucose, 2.0% yeast extract, and 0.1% KH_2PO_4 and adjusted to pH 6.5 with NaOH. The medium was contained in 250-ml. Erlenmeyer flasks (60 ml. per flask) and, after inoculation, incubated for as long as 22 days at 30°C. with and without intermittent agitation. The cellulose pads formed after 4, 7, 10, 14, 18, and 22 days were washed thoroughly with deionized water and dried to a constant weight at 110°C. Cellulose formed by Acetobacter accumulates in a pellicle at the surface of liquid medium. Within 24 hours a light, tenuous pellicle is detectable and, as shown in Fig. 1, cellulose formed at a constant rate for about 18 days to a maximum yield of about 30%. Flasks agitated during incubation were harvested at 22 days only and the results indicated an inhibition of cellulose formation.

TEMPERATURE AND pH EFFECTS

The effects of temperature and pH on cellulose yield, and of pH on viable cell population, in a glucose-yeast extract-KH_2PO_4 medium are shown in Fig. 2 and 3. For the temperature study, the incubation time was 15 days at

FIGURE 1. RATE OF CELLULOSE FORMATION

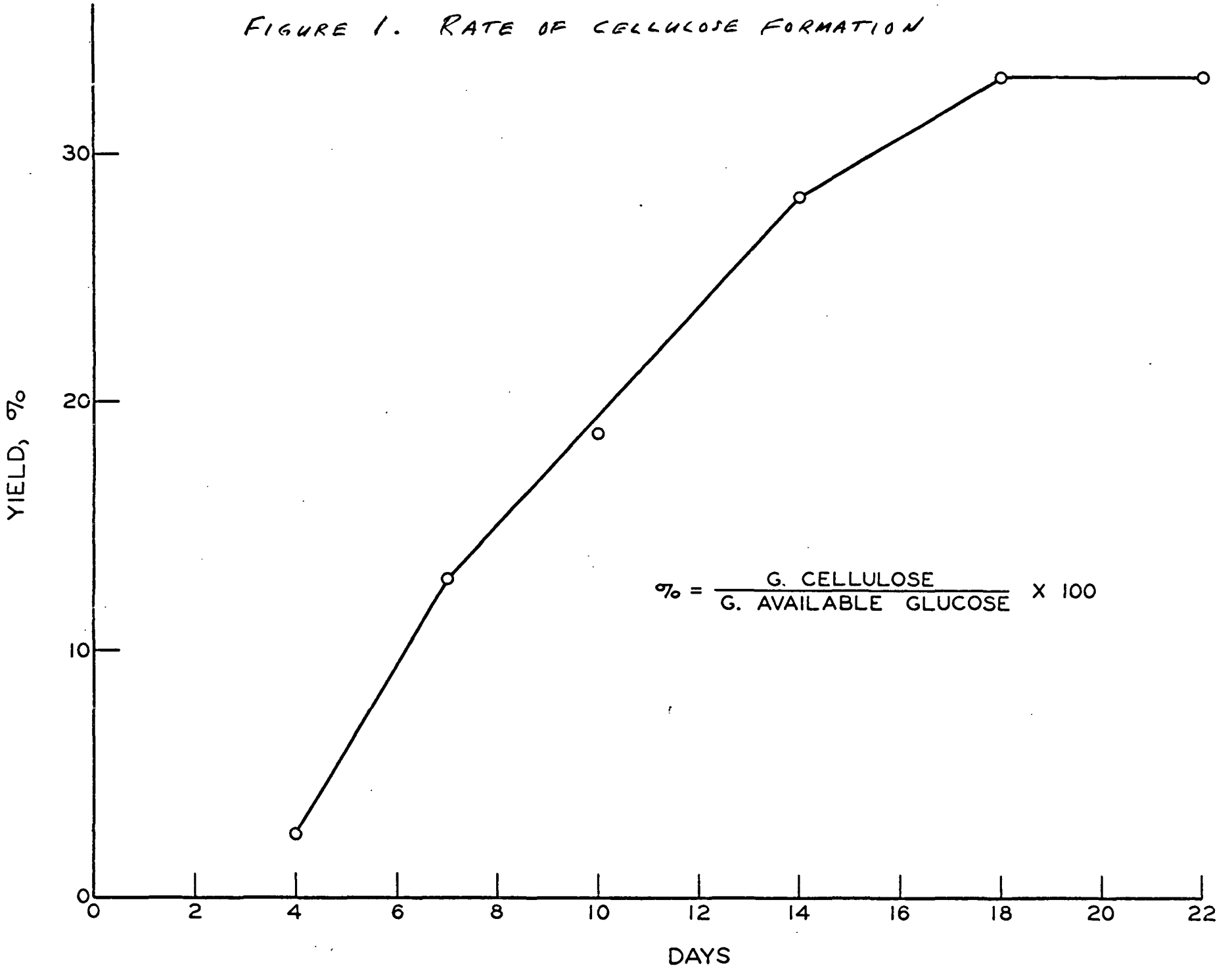
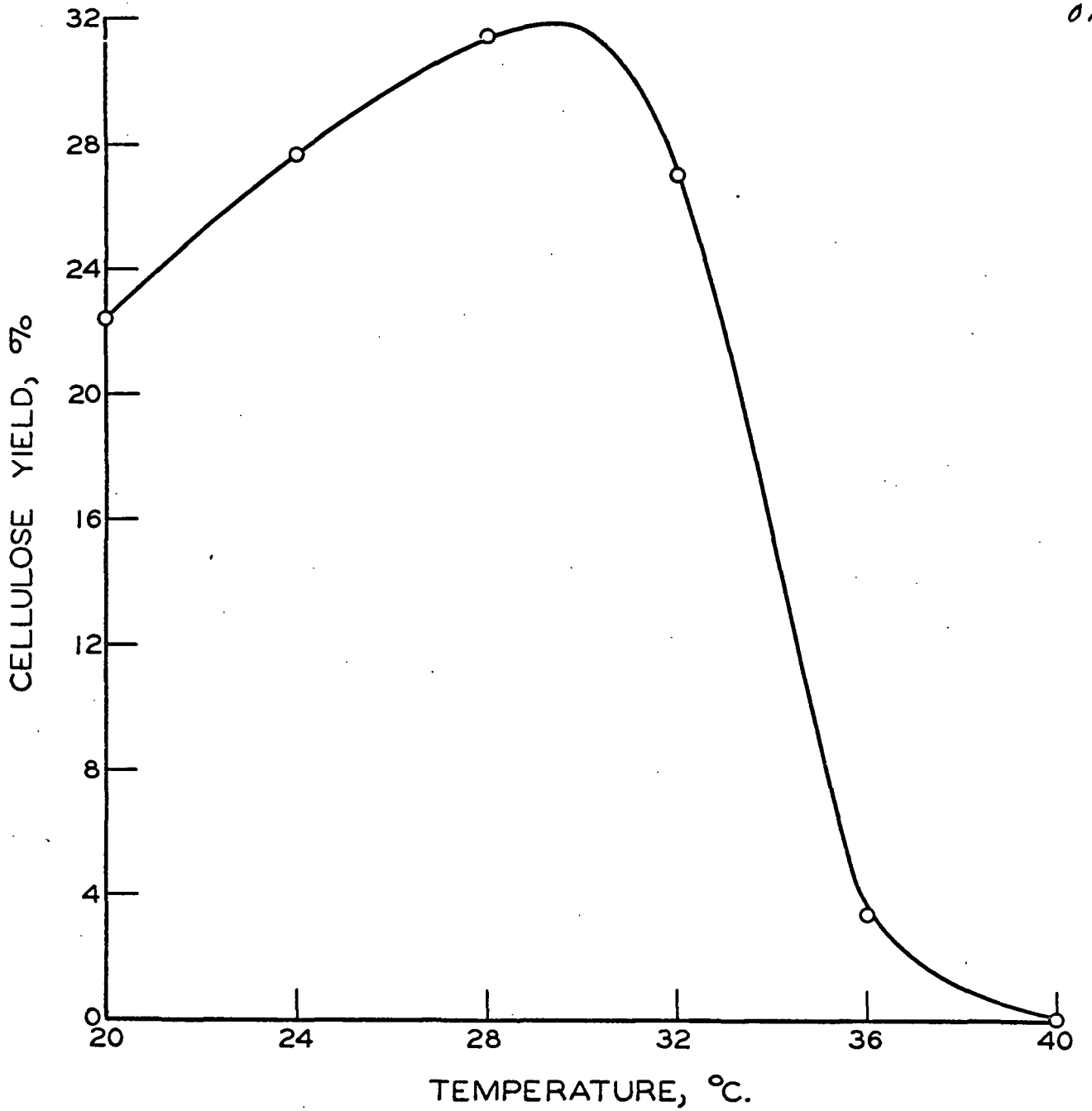


FIGURE 2. EFFECT OF TEMPERATURE ON CELLULOSE YIELD



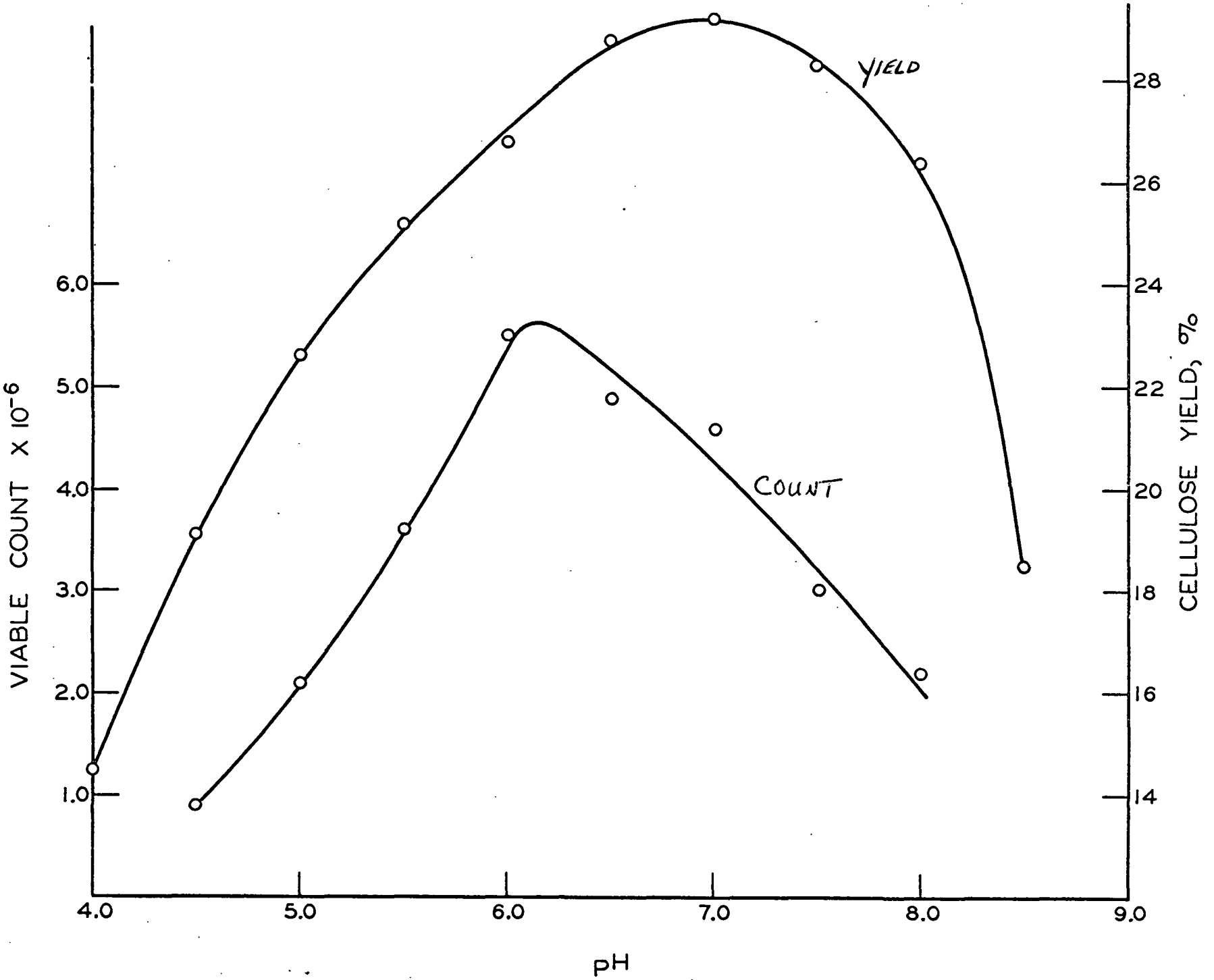


FIGURE 3. pH VERSUS VIABLE COUNT AND CELLULOSE YIELD

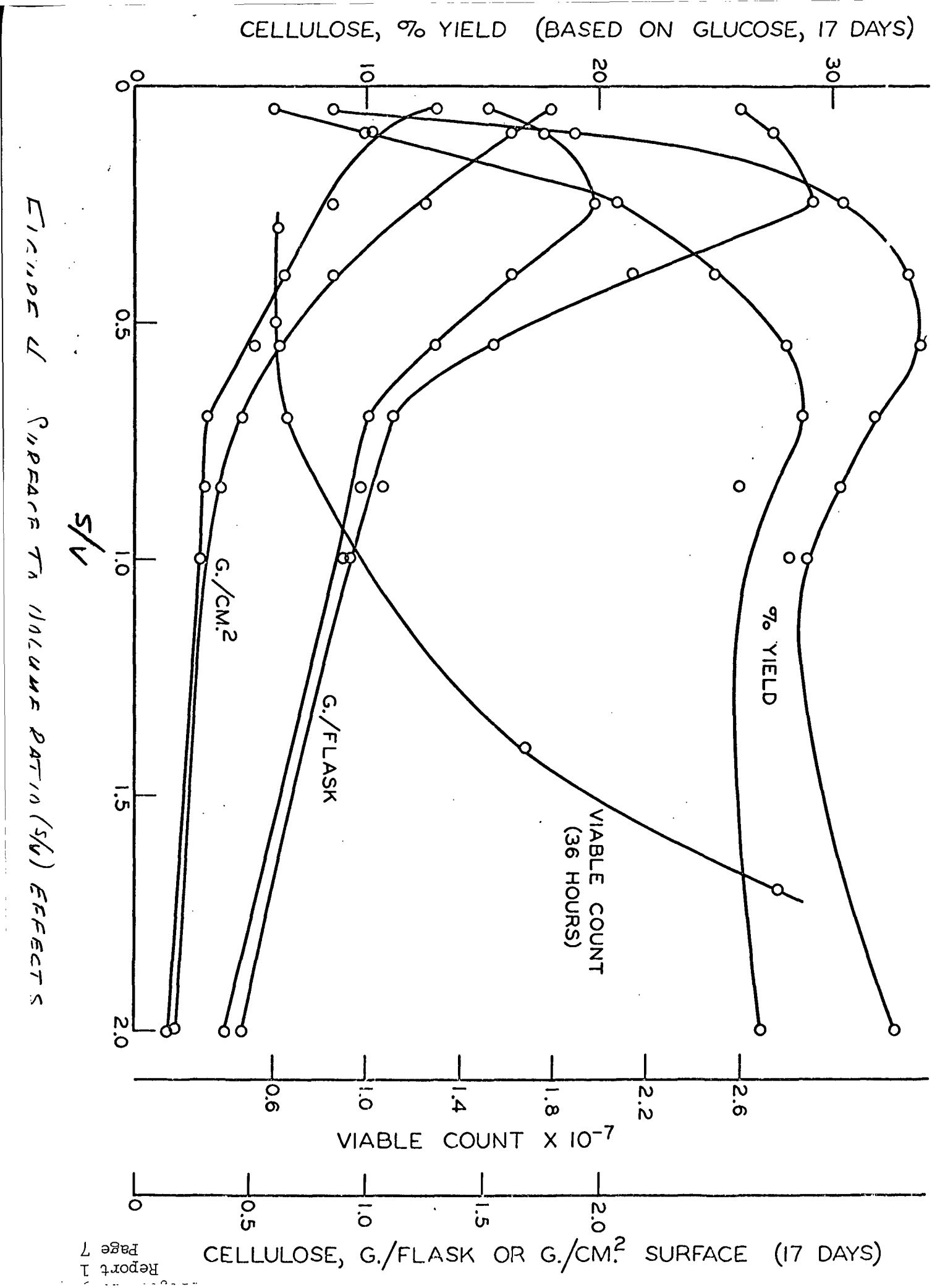
20, 24, 28, 32, 36, and 40°C. Cellulose pads were washed and dried at 110°C. For the pH study, cell counts were made after 30 hours' incubation at 30°. Beyond this time, entanglement of cells in the cellulose pellicle precludes accurate cell counts. Cellulose yield was determined after 17 days' incubation by the usual procedure. The highest cell count was at pH 6.2, whereas the pH optimum for cellulose yield was nearly 7.0. The results also indicate a direct dependence of yield on cell population below pH 6.0; however, the yield per cell at pH 6.0-8.0 increased considerably. Based on these results subsequent experiments were run at 30°C. and pH 6.5.

SURFACE-TO-VOLUME RATIO

Early results on cellulose formation by Acetobacter were erratic and apparently due to the use of various flask sizes and medium volumes for the fermentation. This led to an investigation of the effect of the surface-to-volume ratio on cellulose yield in still cultures. Figure 4 summarizes the results (similar curves represent duplicate experiments). The usual glucose-yeast extract-KH_2PO_4 medium (pH 6.5) was used with viable counts made after 36 hours' incubation (30°C.) and other data determined after 17 days. Limited by oxygen diffusion, cell growth and cellulose formation proceed more slowly in deeper cultures (low surface-to-volume ratios). A maximum yield was observed at an s/v ratio of about 0.5.

RADIOISOTOPE STUDIES

The results of four experiments involving C-14 labeled ethanol, glucose, alanine, aspartic acid, and glutamic acid are presented in Tables I-IV. Experiments 3, 4, and 6 were run in the usual yeast extract medium, while a chemically



EFFECT OF SURFACE TO VOLUME RATIO (S/V) EFFECTS

TABLE I

RESULTS OF EXPERIMENT 3

(Yeast extract medium, incubation 36 days)

Ethanol, %	Glucose, % 0.1			0.5			1.0			1.5			3.0			10.0		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0.0	5	181	1.4	18	288	11	37	320	24	54	306	34	100	227	51	111	36	27
0.3	10	486	3.6	25	411	15	41	372	28	55	342	38	106	270	61	126	39	29
0.9	14	713	5	32	582	22	48	478	36	61	434	49	108	312	70	132	40	30
1.8	14	767	6	36	693	26	55	598	45	70	530	59	112	391	88	141	40	30
3.6	16	813	6	38	740	28	60	659	49	75	585	66	120	480	108	151	39	29
0.0	5	.059		18	.082		39	.098		56	.073		100	.093		118	.126	
0.3	12	13.2	.86	28	16.6	1.08	42	20.5	1.36	57	22.8	1.50	104	28.4	1.86	130	36.2	2.39
0.9	14	11.5	.76	33	15.2	1.01	48	15.8	1.05	64	16.9	1.12	110	21.1	1.40	135	23.6	1.56
1.8	15	6.2	.41	38	9.9	.65	56	12.3	.81	70	13.3	.88	115	15.8	1.05	150	16.7	1.11
3.6	15	3.0	.20	40	5.1	.34	62	7.2	.48	79	8.3	.55	122	10.8	.71	160	11.9	.79
0.0	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
0.3	10	44.7	3.06	25	43.0	2.95	41	36.9	2.53	56	37.0	2.53	104	36.6	2.50	128	42.6	2.92
0.9	14	38.2	2.60	32	40.4	2.75	47	35.6	2.43	62	32.6	2.22	107	27.1	1.84	139	28.4	1.94
1.8	14	19.2	1.30	36	28.0	1.91	55	27.5	1.87	70	25.9	1.76	115	22.8	1.55	154	20.2	1.37
3.6	15	7.7	0.52	38	10.8	0.74	62	13.6	0.93	77	14.9	1.01	121	14.9	1.01	157	13.1	0.89

Note: A = product, mg.; B = C-14, c.p.m. x 10^{-3}; C = incorporation, mg.

Label: Top – Glucose-UL-C-14 (1605 x 10^3 c.p.m. per treatment).

Middle – Ethanol-C-1-14 (1515 x 10^3 c.p.m. per treatment).

Bottom – Ethanol-C-2-14 (1464 x 10^3 c.p.m. per treatment).

TABLE II

RESULTS OF EXPERIMENT 4

(Yeast extract medium, incubation 36 days)

Ethanol, %	Glucose, % 0.1			1.5			3.0			4.5			6.0			7.5			10.0		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0.0	7	98	1.7	56	170	42	104	188	94	110	87	66	101	36	37	113	33	41	114	24	40
0.3	11	211	3	60	209	52	105	214	108	-	-	-	-	-	-	-	-	-	-	-	-
0.9	15	309	6	67	240	60	109	210	106	-	-	-	-	-	-	-	-	-	-	-	-
1.8	15	324	6	71	244	61	112	218	110	168	210	158	160	69	69	159	53	67	156	33	56
3.6	14	332	6	74	269	68	115	242	121	163	222	167	175	104	104	167	54	68	154	31	52
4.5	-	-	-	73	273	69	115	241	121	158	224	169	169	89	89	157	50	62	109	20	33
5.4	-	-	-	70	251	63	113	236	119	159	225	170	174	118	119	147	50	62	103	19	32
6.3	-	-	-	67	265	67	112	233	117	156	233	176	159	93	93	125	34	42	77	14	23
7.2	-	-	-	69	263	66	111	250	126	156	225	170	170	126	127	116	36	46	91	21	36
8.1	-	-	-	72	299	76	115	255	128	144	183	138	121	53	53	119	40	50	-	-	-

A = Product, mg.

B = C-14, c.p.m. x 10^{-3}.

C = Glucose incorporation, mg. (based on 1605 x 10^{3} c.p.m. per treatment).

TABLE III

RESULTS OF EXPERIMENT 6

(Yeast extract medium, incubation 21 days)

Ethanol, %	Glucose, % 1.5			3.0			4.5			6.0			7.5			10.0		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0.0	45	346	52	85	320	96	104	270	121	97	192	115	95	149	112	97	110	110
1.8	59	419	63	97	364	110	144	350	158	158	293	175	142	206	154	124	137	148
4.5	59	430	64	97	366	110	127	315	152	119	226	135	103	163	122	66	79	79
6.3	50	366	54	70	276	83	63	169	75	52	104	62	41	73	54	24	35	34
8.1	37	300	45	34	146	43	26	82	37	33	76	45	24	47	35	15	26	25

A = product, mg.

B = C-14, c.p.m. x 10^{-3}.

C = glucose incorporation, mg. (based on 1200 x 10^3 c.p.m. per treatment).

TABLE IV

RESULTS OF EXPERIMENT 7

(Synthetic medium,[a] incubation 36 days)

Glucose, %	Product, mg.				C-14, c.p.m. x 10^{-3}				Incorporation, mg.			
	GL	ALA	ASP	GLUT	GL	ALA	ASP	GLUT	GL	ALA	ASP	GLUT
0.1	7	7	6	6	304	252	5.0	6.7	4	3.65	0.06	0.09
0.5	18	19	9	10	198	129	1.6	1.5	13	1.87	0.02	0.02
1.0	25	25	17	14	216	103	2.4	1.8	28	1.49	0.03	0.02
1.5	36	35	29	26	208	74	1.5	1.7	41	1.07	0.02	0.02
3.0	45	42	33	27	137	8.7	0.8	1.4	54	0.13	0.01	0.02
4.5	54	65	48	59	107	6.7	1.0	1.7	63	0.10	0.01	0.02
6.0	98	98	90	86	139	10.7	1.7	2.1	110	0.15	0.02	0.03
7.5	70	81	73	71	80	8.2	1.5	1.9	79	0.12	0.02	0.03
10.0	66	77	71	62	56	6.6	1.4	1.8	74	0.10	0.02	0.02

GL = glucose (912 x 10^3 c.p.m. per treatment).

ALA = alanine (1518 x 10^3 c.p.m. per treatment).

ASP = aspartic acid (1182 x 10^3 c.p.m. per treatment).

GLUT = glutamic acid (1572 x 10^3 c.p.m. per treatment).

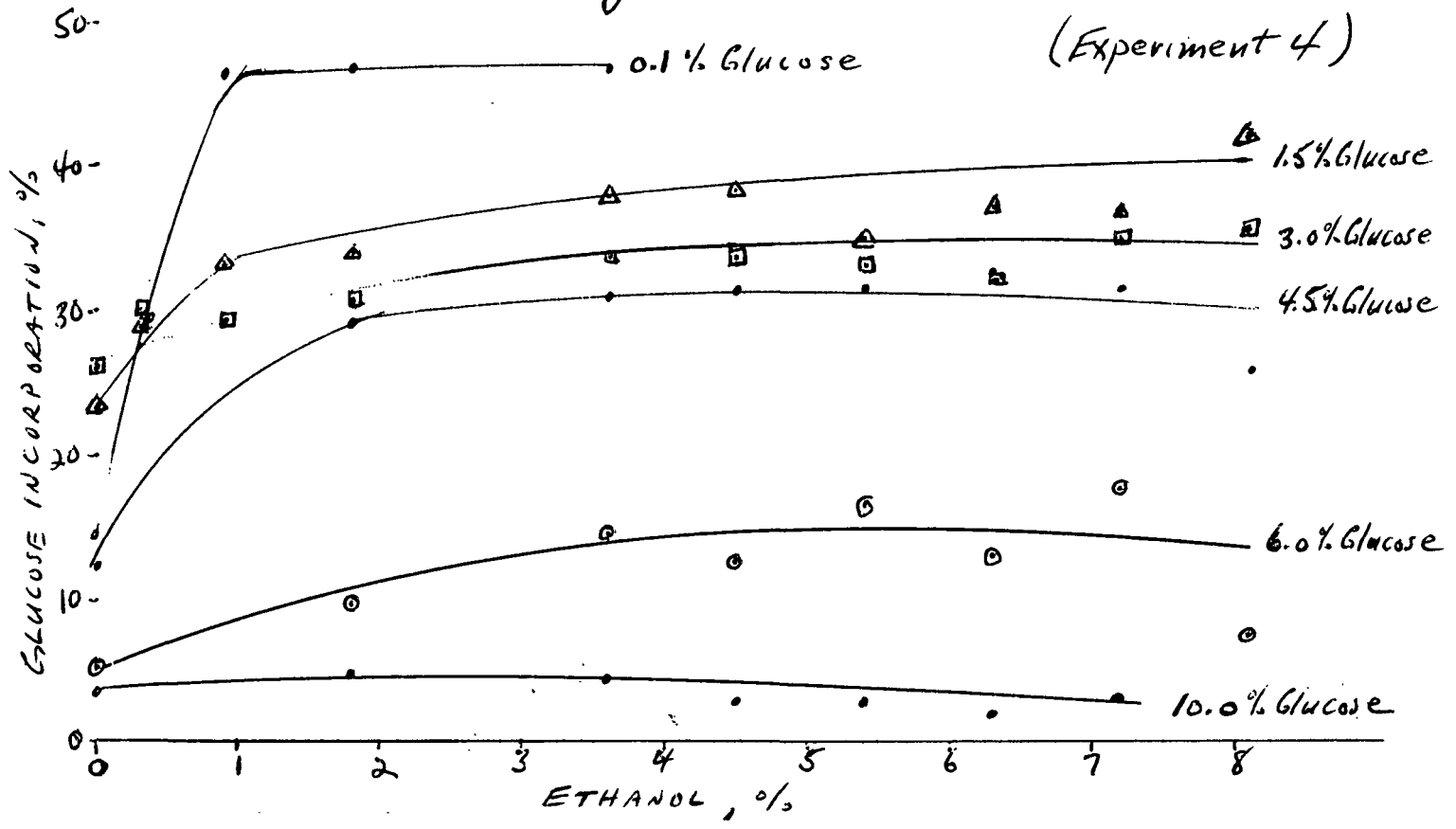
[a] Synthetic or defined medium:

	g./liter		g./liter
KH_2PO_4	2.0	Glutamic acid	2.0
$MgSO_4$ $7H_2O$	1.0	Ca D-pantothenate	0.002
$FeSO_4$ $7H_2O$	0.01	Riboflavin	0.002
$(NH_4)_2HPO_4$	2.5	Biotin	0.0001
p-Aminobenzoic acid	0.01	Glucose	variable
Alanine	2.0	Ethanol	(3.2%)
Aspartic acid	1.2		

pH adjusted to 6.0 with NaOH; sterilization by Millipore filter.

defined medium was used for Experiment 7. In all cases the medium was contained in 17 x 150-mm. test tubes, 4.0 ml. per tube (s/v 0.55). Each glucose:ethanol combination was replicated in three tubes; however, at the end of the incubation period the cellulose pads in triplicate tubes were combined for freeze-dry weight determinations and radioactivity measurements. A double-strength medium solution was inoculated from a culture of Acetobacter grown on an agar slant (2.0% glucose, 0.5% peptone, 0.5% yeast extract, 1.5% agar). Growth from the slant, which had been incubated 3 days, was suspended in about 20 ml. of water and 1.0 ml. was added to the double-strength medium. Also incorporated in the medium was an appropriate level of C-14-labeled substrate. The medium was then dispensed to sterile test tubes, 2.0 ml. per tube. Each tube also received 1.0 ml. of a previously autoclaved glucose solution and 1.0 ml. of a Millipore-filtered ethanol solution to give desired final concentrations in 4.0 ml. Incubation was at 30°C. for 21 or 36 days, after which the cellulose pads were washed with water, autoclaved in 0.5N NaOH for 60 minutes, treated with 1.0% acetic acid, washed again with water, and finally freeze-dried. After weighing, the pads were placed in a scintillation counting solution (Cocktail D: 100 g. naphthalene and 5.0 g. 2,5-diphenyloxazole per liter of dioxane) and counted in a Beckman liquid scintillation system using the full C-14 window. Values of glucose, ethanol, and amino acid incorporation by weight, tabulated in Tables I-IV, were determined from C-14 counts on the cellulose product relative to the specific activity of the substrate present in the medium. Since a uniform level of label was added to each test tube in a given experiment, specific activities varied with substrate concentration. Percentage incorporation figures were calculated (% = mg. incorporation/mg. substrate available x 100) and plotted as a function of ethanol concentration (Fig. 5 and 8) and as a function of glucose concentration (Fig. 6, 7, and 9). The results show a decreasing percentage

Figure 5


(Experiment 4)
0.1 % Glucose
1.5% Glucose
3.0% Glucose
4.5% Glucose
6.0% Glucose
10.0% Glucose
GLUCOSE INCORPORATION, %
ETHANOL, %
0
10
20
30
40
50
0
1
2
3
4
5
6
7
8

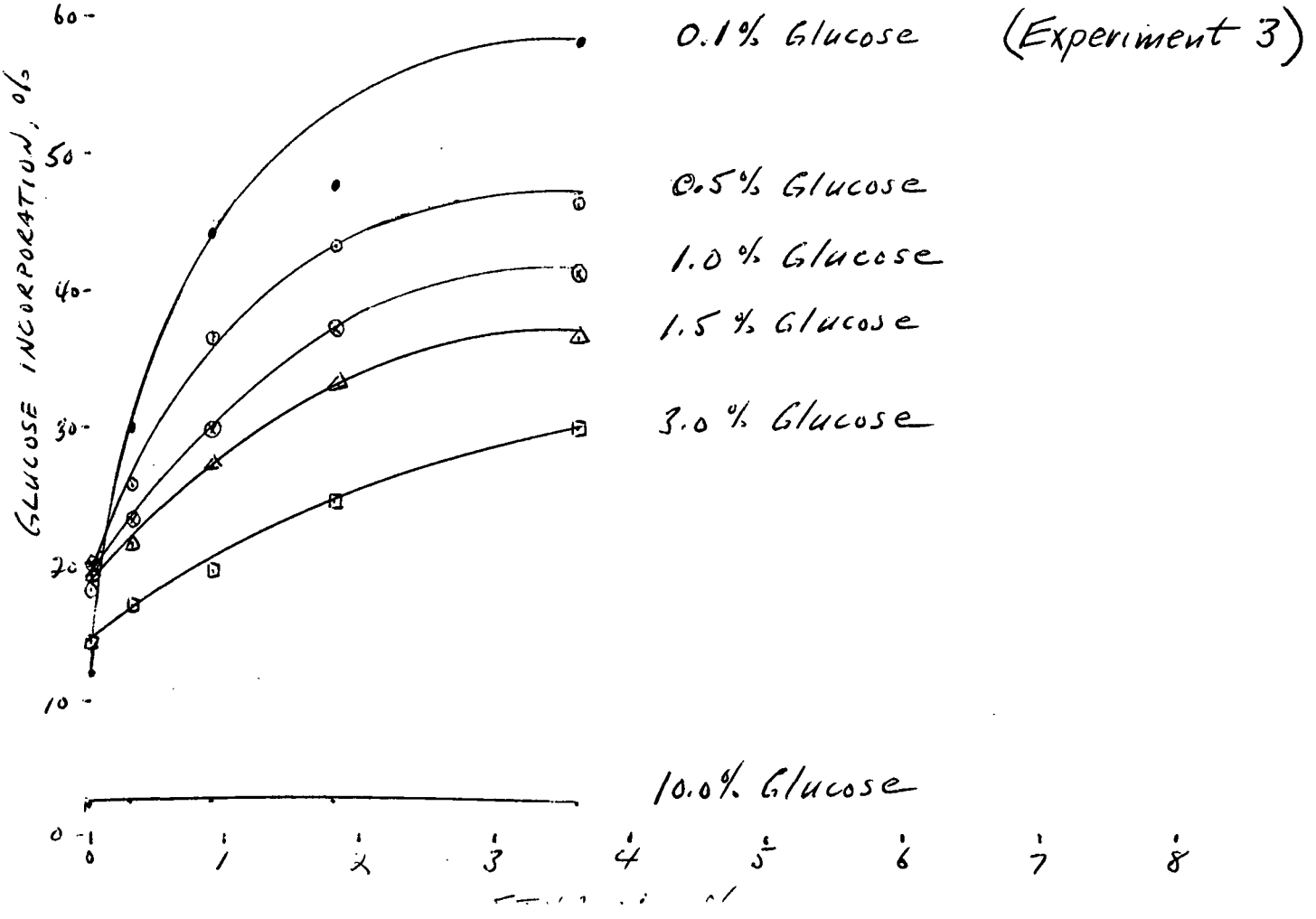

(Experiment 3)
0.1% Glucose
0.5% Glucose
1.0 % Glucose
1.5 % Glucose
3.0 % Glucose
10.0% Glucose
GLUCOSE INCORPORATION, %
10
20
30
40
50
60
0
1
2
3
4
5
6
7
8

Figure 6

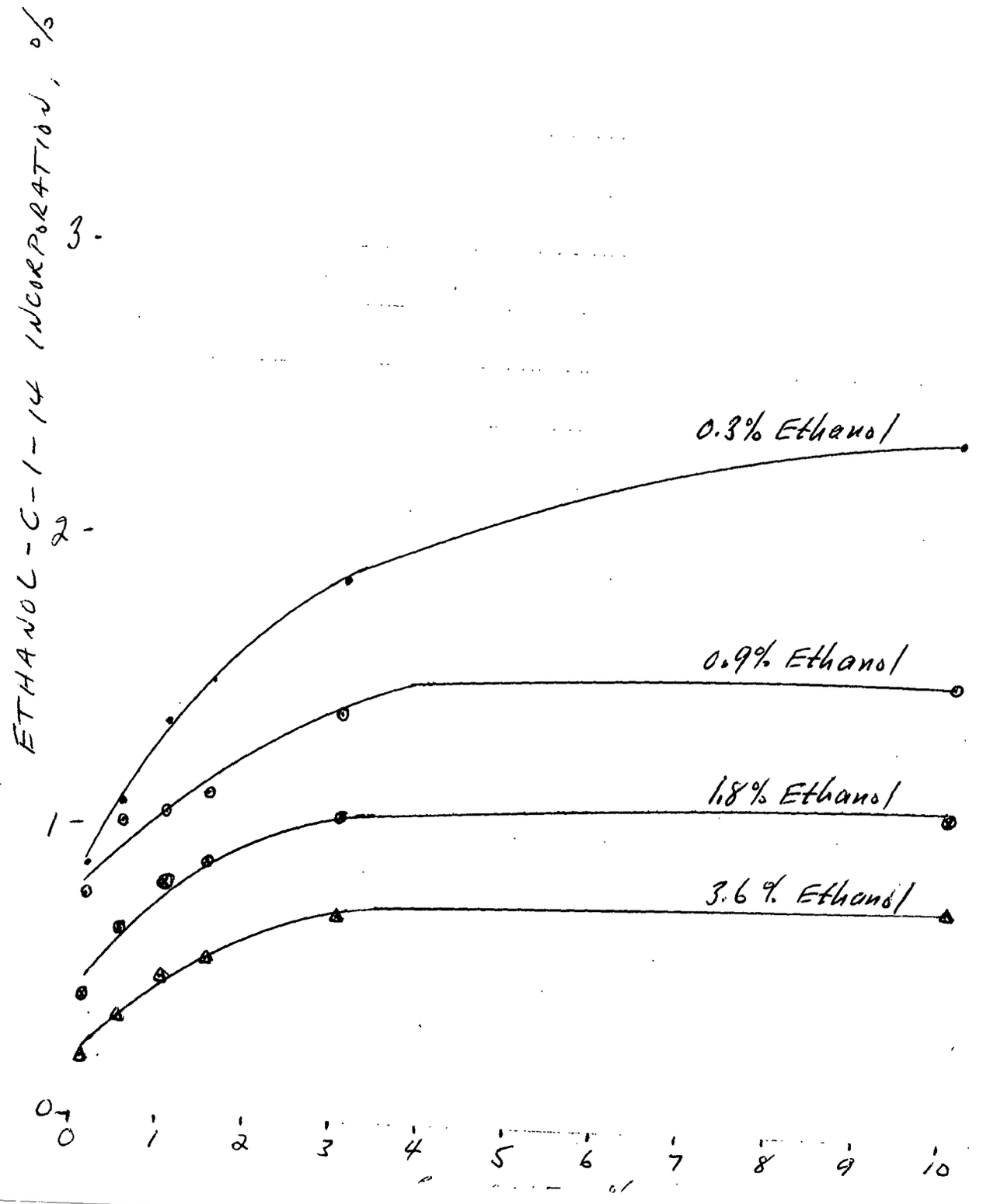

ETHANOL-C-1-14 INCORPORATION, %
3.
2.
1.
0
0.3% Ethanol
0.9% Ethanol
1.8% Ethanol
3.6 % Ethanol
0
1
2
3
4
5
6
7
8
9
10

Figure 7

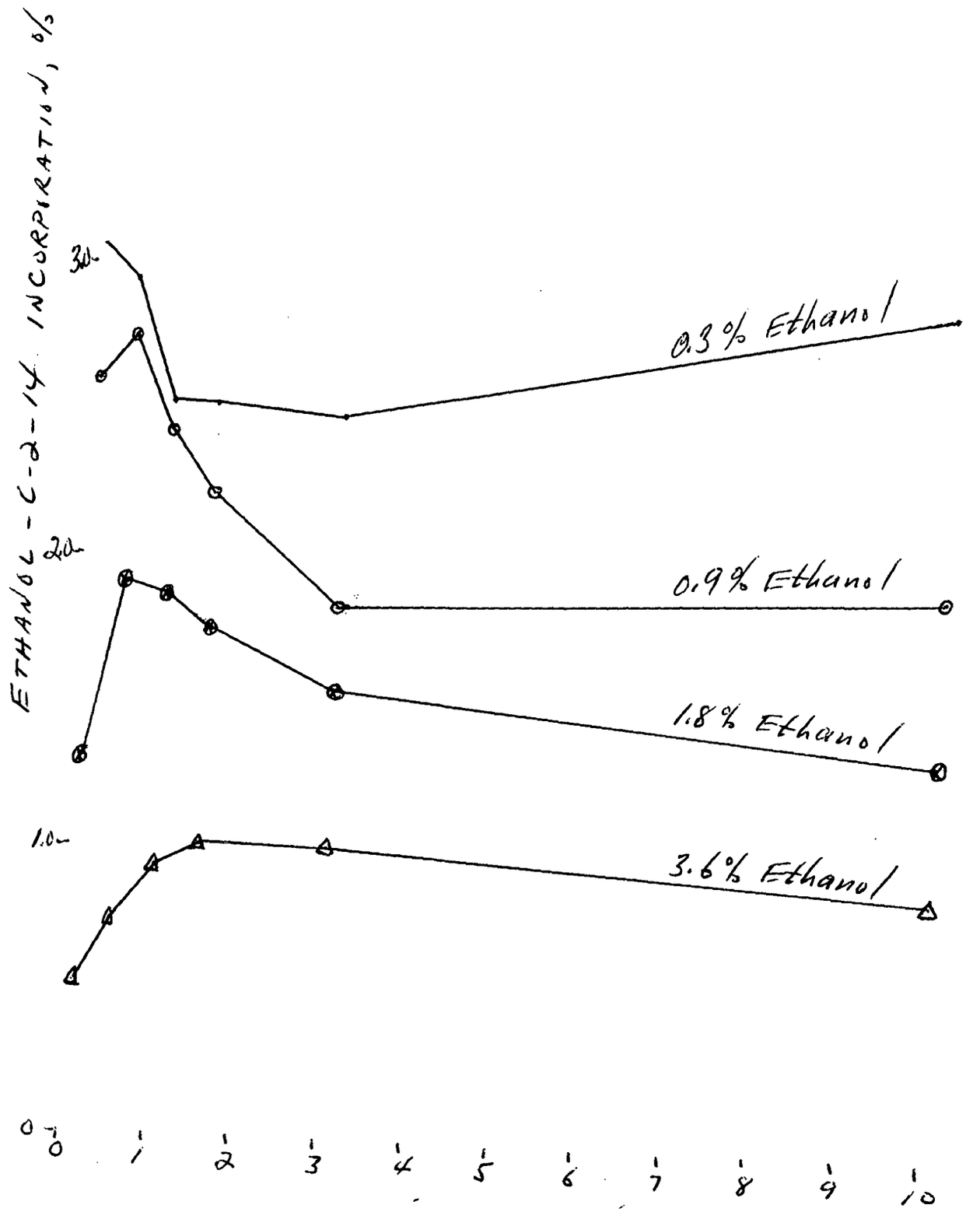

ETHANOL-C-2-14 INCORPORATION, %
30
20
10
0
0.3% Ethanol
0.9% Ethanol
1.8% Ethanol
3.6% Ethanol
0
1/10
1/9
1/8
1/7
1/6
1/5
1/4
1/3
1/2
1/1

Figure 8

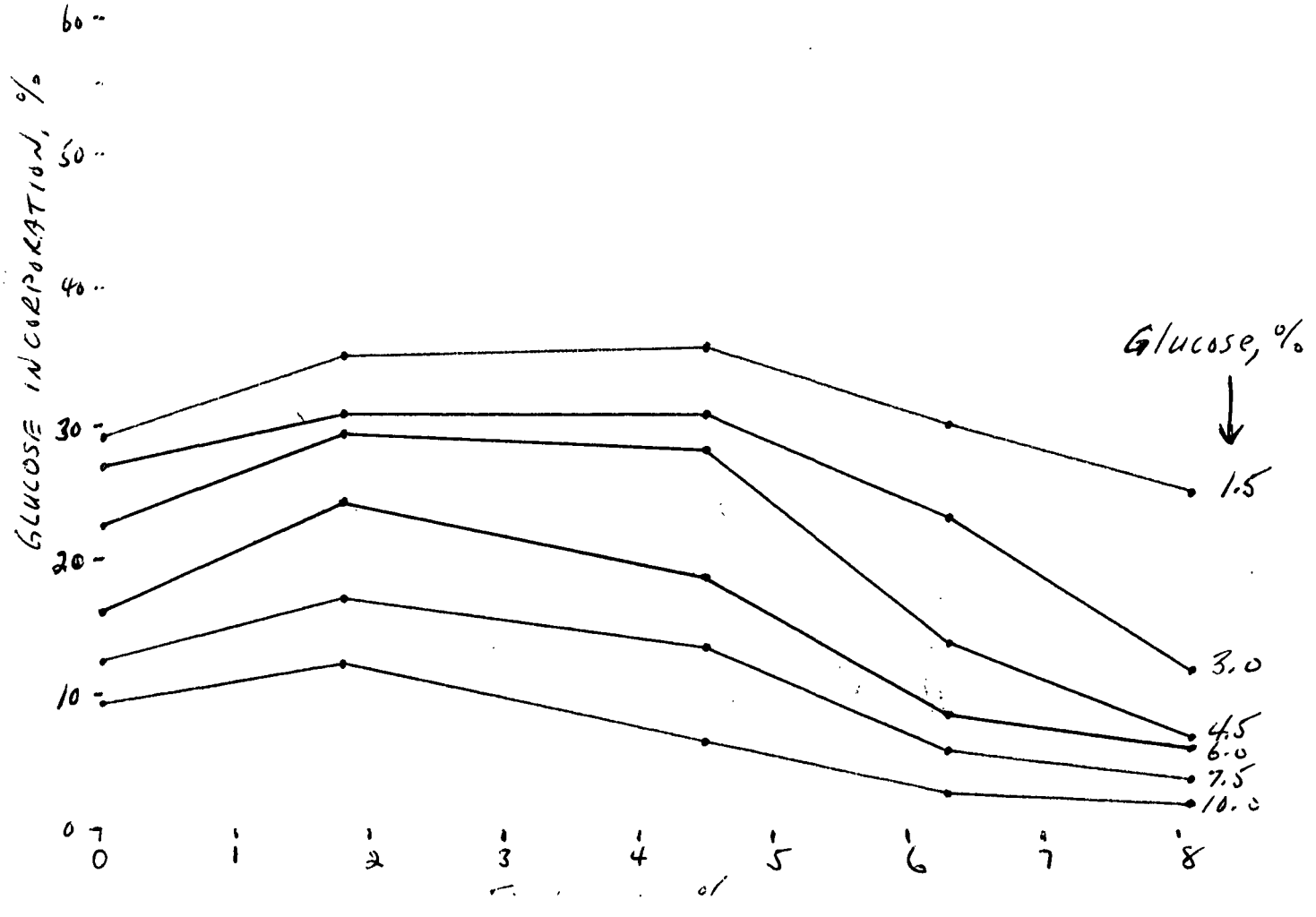
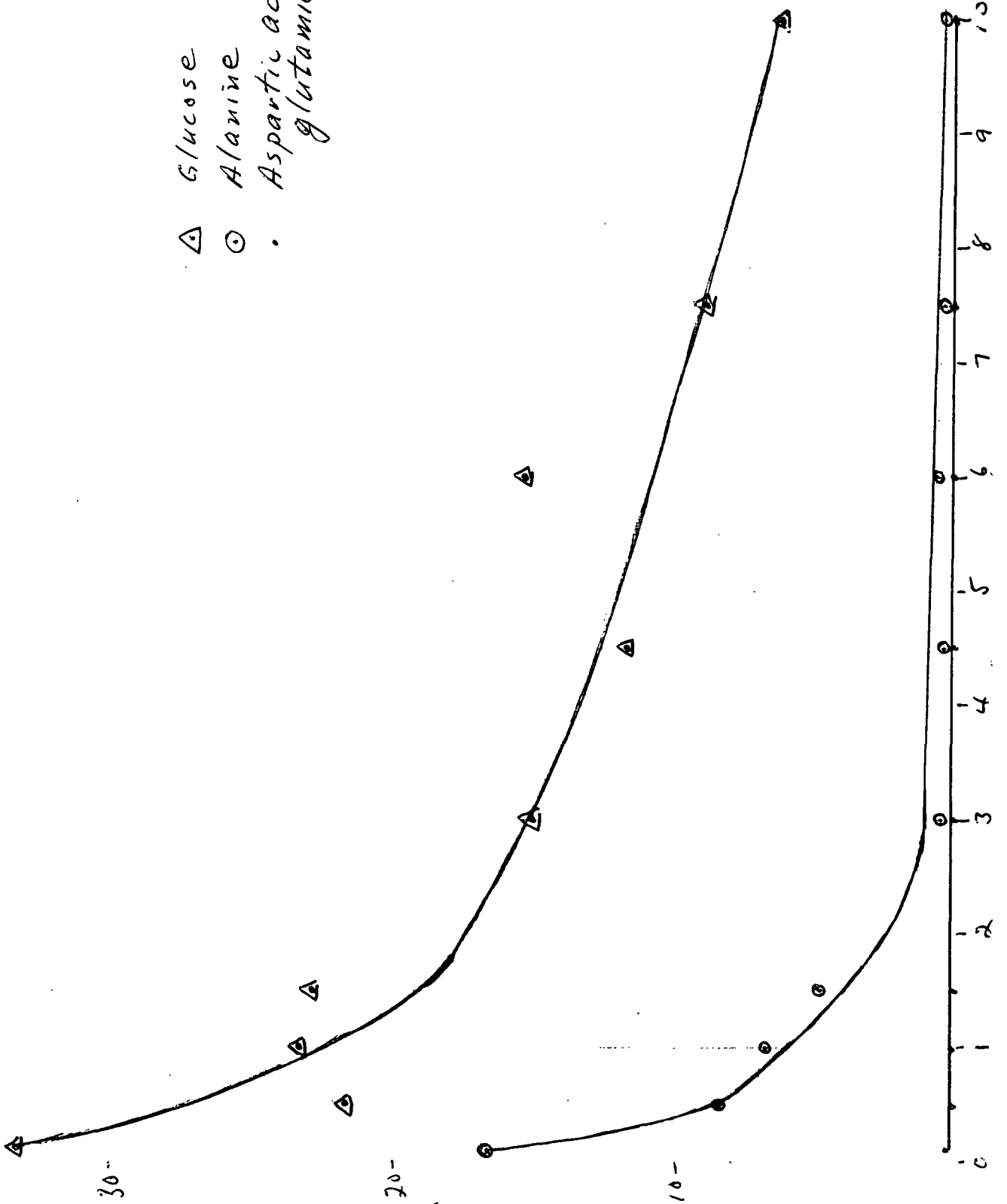

GLUCOSE INCORPORATION, %
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
Glucose, %
1.5
3.0
4.5
6.0
7.5
10.0

Figure 9


Δ Glucose
○ Alanine
• Aspartic acid and glutamic acid.
INCUBATION, %
30
20
10
0
1
2
3
4
5
6
7
8
9
10

conversion of glucose to cellulose as the concentration of glucose in the medium is increased. This was true at all levels of ethanol (0-8.1%) as shown in Fig. 5 and 8. Percent glucose incorporation increased greatly, especially at low glucose levels, at ethanol concentrations up to about 1.0%; however, increased ethanol concentration either had no effect (Fig. 5) or glucose incorporation decreased (Fig. 8). Although the presence of ethanol in the growth medium influences the level of glucose incorporation into cellulose, the utilization of ethanol-C-1-14 and ethanol-C-2-14 was negligible (Fig. 6 and 7). Of the three amino acids only alanine, at low levels of glucose, was significantly incorporated (Fig. 9).

Experiments similar to those described above were run to investigate the utilization of 2-deoxy-2-fluoro-D-glucose, 2-deoxy-D-glucose, and D-mannose by Acetobacter. 2-Deoxy-2-fluoro-D-glucose and 3-deoxy-D-glucose were prepared by Paul Seib. 2-Deoxy-D-glucose and D-mannose were purchased from Pfanstiehl Chemicals, Waukegan, Illinois. The compounds were incorporated in yeast extract medium at concentrations of 0.0, 0.1, 0.25, 0.5, 1.5, 2.5, 3.5, 3.92, and 4.0% with corresponding glucose concentrations of 4.0, 3.9, 3.75, 3.5, 2.5, 1.5, 0.5, 0.08, and 0.0%. Incubation was at 30°C. for 14 days and processing of cellulose pads was done as previously described except that the autoclaving step was omitted. Glucose incorporation results are presented in Table V, amino acid incorporation results in Table VI, and 2-deoxy-D-glucose and D-mannose utilization in Table VII. Percent incorporation as a function of glucose concentration is shown in Fig. 10, 11, and 12. The results indicate an inhibition of glucose and amino acid incorporation in the presence of all four sugars as compared to the glucose-only control. Also, the levels of 2-deoxy-D-glucose-UL-C-14 and

TABLE V

GLUCOSE INCORPORATION INTO CELLULOSE BY Acetobacter IN THE PRESENCE OF THREE SUGAR DERIVATIVES AND D-MANNOSE

Glucose, %	Other Sugar, %	Control			2-F-G			2-Deoxy			3-Deoxy			D-Mannose		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0.00	4.00	3.7	--	--	1.6	--	--	1.3	--	--	2.0	--	--	6.4	--	--
0.08	3.92	6	722	1.7	0.4	27	0.06	0.9	54	0.13	5.4	441	1.0	7.2	374	0.9
0.50	3.50	16	667	10	1.8	66	0.10	5.9	175	2.6	14	578	8.6	13	436	6.5
1.50	2.50	28	458	24	8.6	130	5.8	12	166	7.4	27	430	19	21	335	15
2.50	1.50	39	390	29	19	179	13	22	231	17	37	361	27	26	245	18
3.50	0.50	47	320	33	31	230	24	31	228	24	45	302	31	33	224	23
3.75	0.25	47	319	36	37	247	28	35	240	27	46	285	32	38	262	29
3.90	0.10	47	288	33	40	241	28	41	272	32	47	282	33	42	265	31
4.00	0.00	48	290	34	--	--	--	--	--	--	--	--	--	--	--	--

Control = other sugars not present in medium (glucose only).
2-F-G = 2-deoxy-2-fluoro-D-glucose.
2-Deoxy = 2-deoxy-D-glucose.
3-Deoxy = 3-deoxy-D-glucose.

A = product, mg.
B = c.p.m. x 10^{-3}.
C = glucose incorporation, mg.

Label: D-glucose-C-14 (1.344 x 10^6 c.p.m. per concentration).

Medium: Yeast extract, 2.0%.
KH_2PO_4, 0.1%.
Ethanol, 1.8%.
Glucose, variable.
Other sugars, variable.

TABLE VI

AMINO ACID INCORPORATION INTO CELLULOSE BY *Acetobacter* IN THE PRESENCE OF THREE SUGAR DERIVATIVES AND D-MANNOSE

Glucose, %	Other Sugar, %	Control			2-F-G			2-Deoxy			3-Deoxy			D-Mannose		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
0.00	4.00	3	30.2	3.0	0.6	3.9	0.4	0.7	5.8	0.6	2.0	13.9	1.4	5.4	26.6	2.6
0.08	3.92	6	36.4	3.6	0.7	4.8	0.5	1.1	7.7	0.8	4.6	23.5	2.3	7.6	30.7	3.0
0.50	3.50	15	41.0	4.1	2.2	7.6	0.7	3.8	13.8	1.4	14	35.5	3.5	11	29.8	3.0
1.50	2.50	27	43.6	4.3	5.4	12.0	1.2	12	23.9	2.4	26	43.1	4.3	17	27.5	2.7
2.50	1.50	38	48.8	4.8	19	29.6	2.9	22	28.9	2.9	35	42.8	4.2	21	29.0	2.9
3.50	0.50	40	43.6	4.3	26	29.1	2.9	31	31.3	3.1	39	40.5	4.0	24	30.3	3.0
3.75	0.25	38	40.0	4.0	31	34.0	3.4	32	32.8	3.2	38	41.5	4.1	28	31.1	3.1
3.90	0.10	39	37.9	3.7	33	34.2	3.4	34	34.0	3.4	37	38.9	3.9	32	34.6	3.4
4.00	0.00	37	40.0	4.0	--	--	--	--	--	--	--	--	--	--	--	--

Control = other sugars not present in medium (glucose only).
2-F-G = 2-deoxy-2-fluoro-D-glucose.
2-Deoxy = 2-deoxy-D-glucose.
3-Deoxy = 3-deoxy-D-glucose.

A = product, mg.
B = c.p.m. x 10^{-3}.
C = amino acid incorporation, %.

Label: Amino acid mix-UL-C-14 (1.01×10^6 c.p.m. per treatment).

Medium: Yeast extract, 2.0%.
KH_2PO_4, 0.1%.
Ethanol, 1.8%.
Glucose, variable.
Other sugars, variable.

TABLE VII

UTILIZATION OF 2-DEOXY-D-GLUCOSE AND D-MANNOSE BY Acetobacter

Glucose, %	2-Deoxy or D-Mannose, %	2-Deoxy			D-Mannose		
		A	B	C	A	B	C
0.00	4.00	0.4	0.52	0.05	6.3	39.1	1.77
0.08	3.92	1.3	0.77	0.04	8.2	35.8	1.62
0.50	3.50	3.4	1.39	0.09	12	27.0	1.22
1.50	2.50	12	3.77	0.23	18	13.3	0.60
2.50	1.50	21	4.27	0.27	22	9.1	0.42
3.50	0.50	30	4.25	0.25	24	7.5	0.35
3.75	0.25	32	4.03	0.30	27	9.3	0.40
3.90	0.10	35	4.23	0.25	31	14.0	0.50

2-Deoxy: 2-deoxy-D-glucose-UL-C-14 (1.61×10^6 c.p.m. per tube).

D-Mannose-UL-C-14 (2.21×10^6 c.p.m. per tube).

A: Product, mg.
B: c.p.m. $\times 10^{-3}$.
C: Incorporation, mg.

Medium: Yeast extract, 2.0%.
KH_2PO_4, 0.1%.
Ethanol, 1.8%.
Glucose, variable.
2-Deoxy or D-mannose, variable.

FIGURE 10

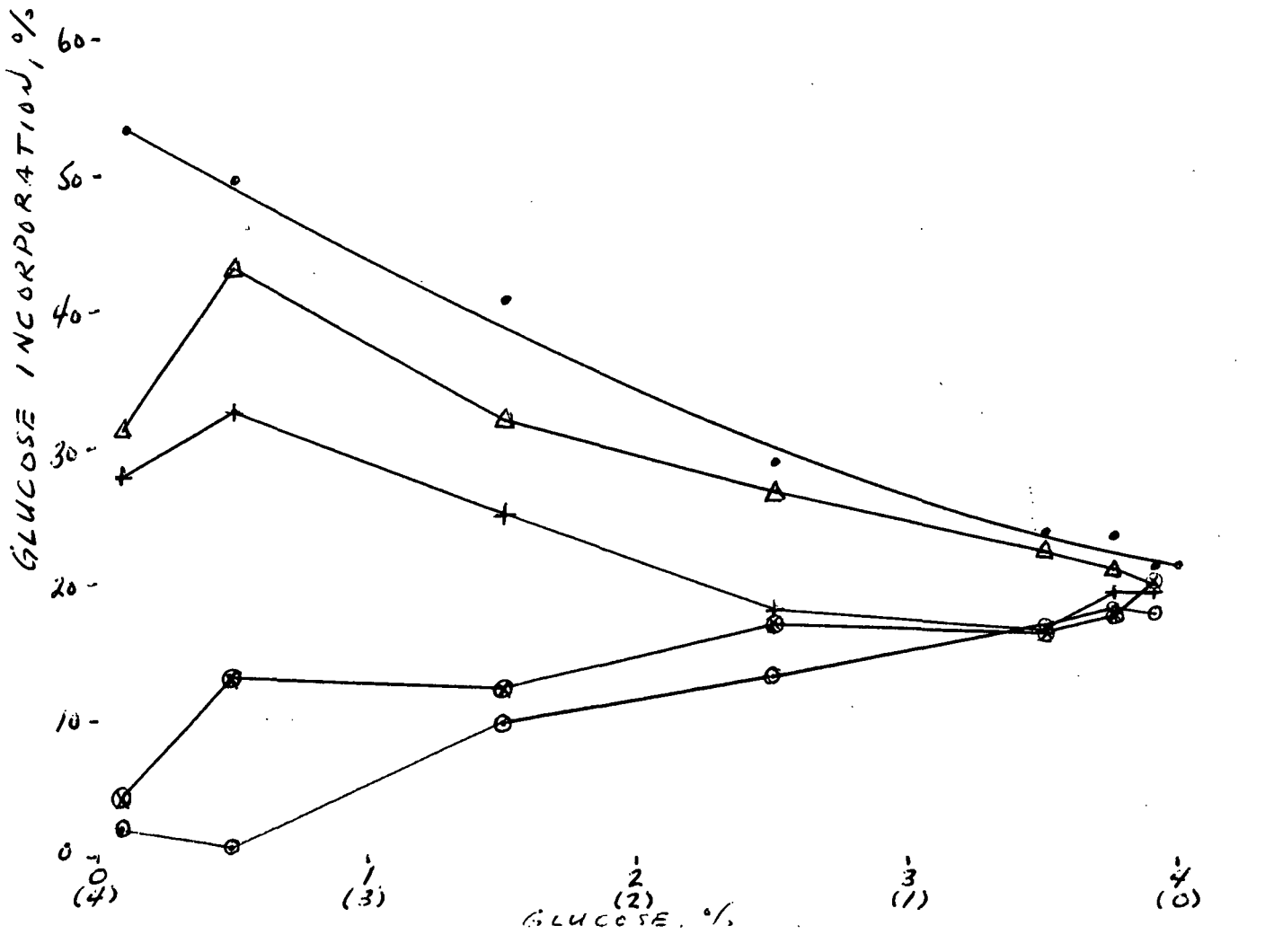

Control
2-Deoxy-2-fluoro-D-glucose
2-Deoxy-D-glucose
3-Deoxy-D-glucose
D-Mannose
GLUCOSE INCORPORATION, %
60
50
40
30
20
10
0
0 (4)
1 (3)
2 (2)
3 (1)
4 (0)
GLUCOSE, %

FIGURE 11

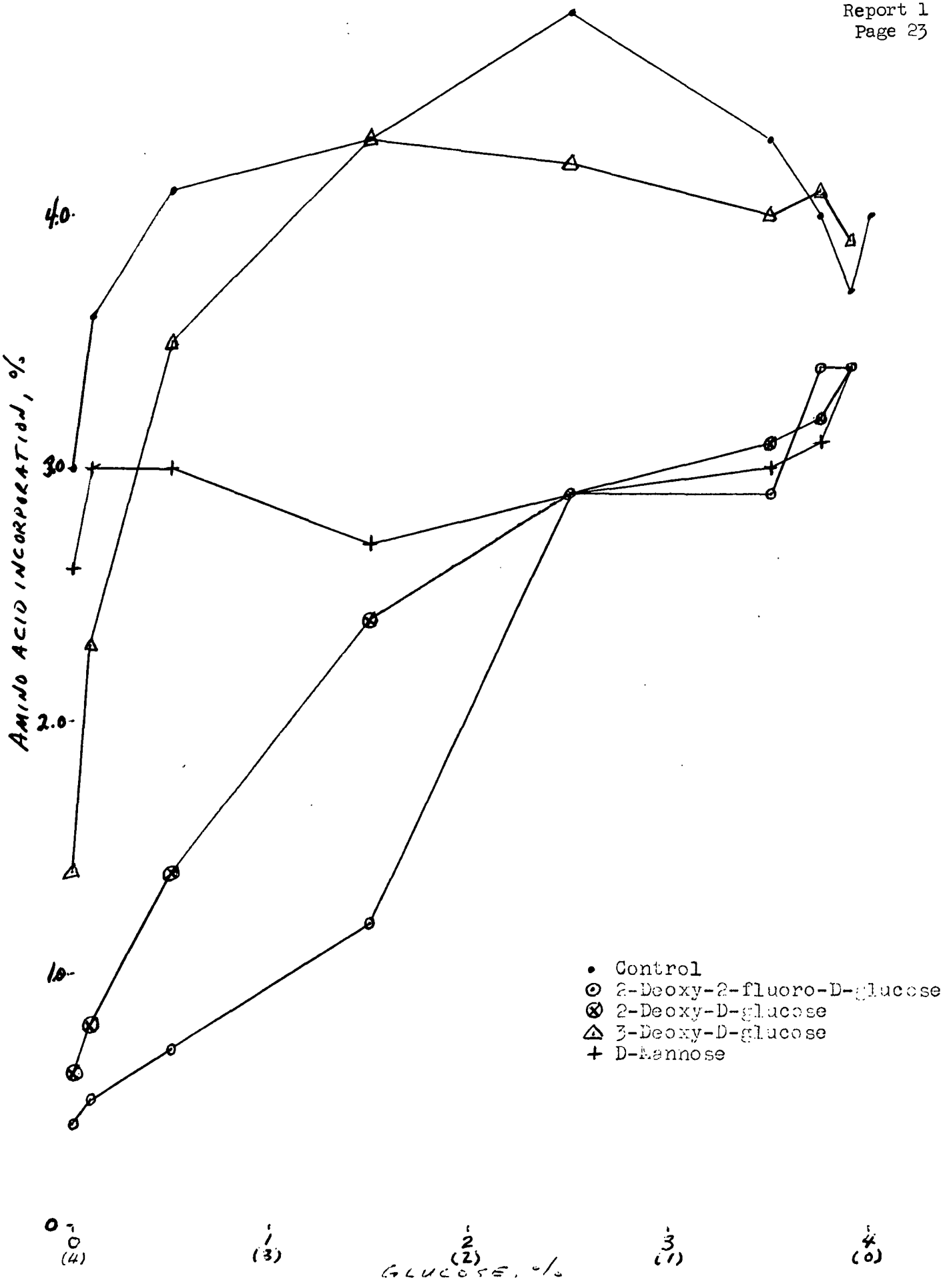
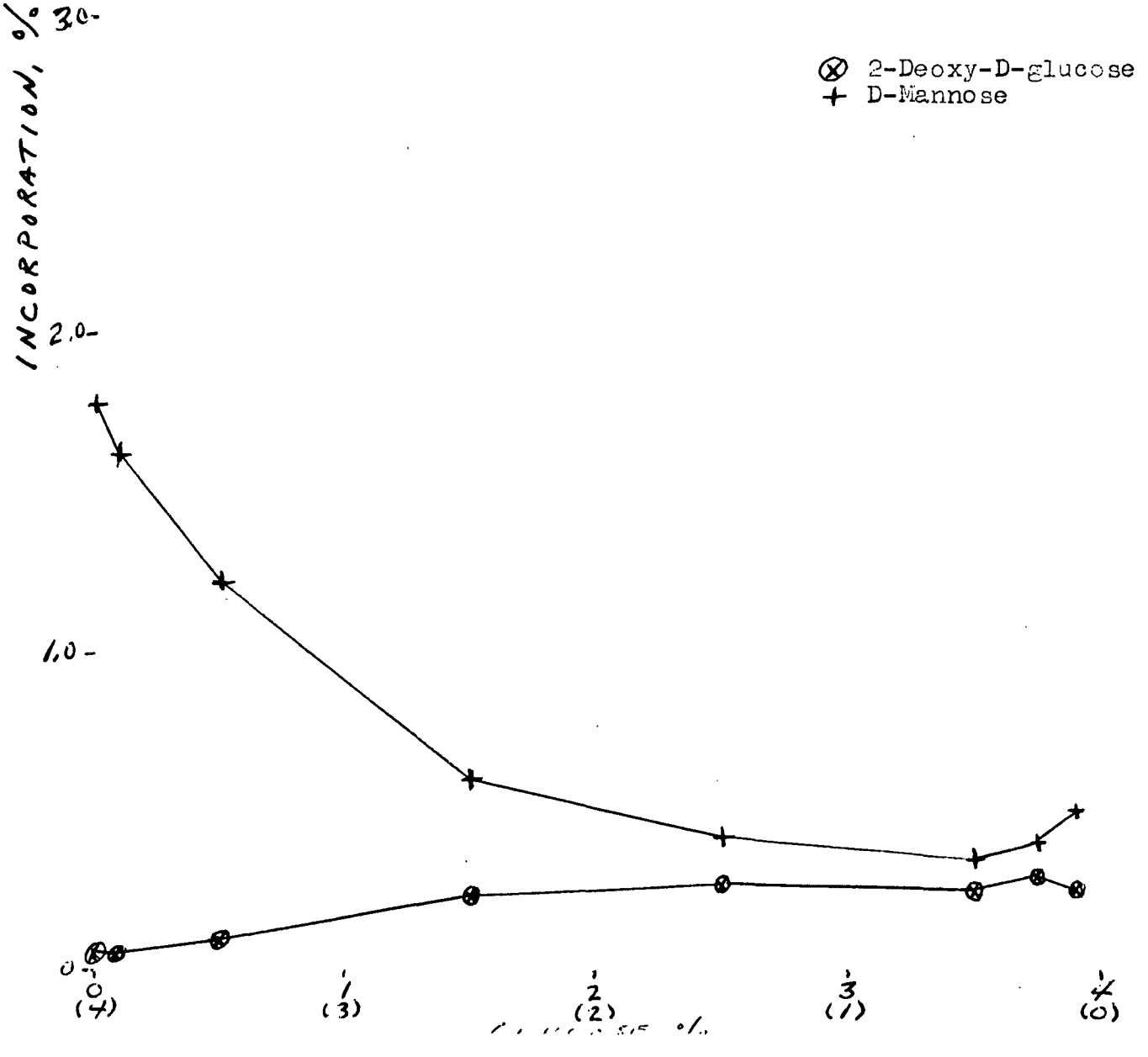

AMINO ACID INCORPORATION, %
4.0
3.0
2.0
1.0
0
0 (4)
1 (3)
2 (2)
3 (1)
4 (0)
GLUCOSE, %
Control
2-Deoxy-2-fluoro-D-glucose
2-Deoxy-D-glucose
3-Deoxy-D-glucose
D-Mannose

FIGURE 12


INCORPORATION, %
3.0
2.0
1.0
0
2-Deoxy-D-glucose
D-Mannose
0 (4)
1/4 (3)
2/4 (2)
3/4 (1)
4/4 (0)

D-mannose-UL-C-14 incorporation were found to be less than 2.0% (Fig. 12). Cellulose pads produced in the presence of the four compounds were hydrolyzed and analyzed by paper chromatography. No significant amount of the three sugar derivatives of D-mannose were detected.

BACTERIAL CELLULOSE AS AN ADDITIVE TO PAPER

Weyerhaeuser bleached sulfite pulp (390 g.) was soaked in tap water for 30 minutes and slowly poured into a Valley beater previously filled with water to the 17-liter mark. After 10 minutes' preliminary slushing, the pulp was beaten to a Schopper-Riegler freeness of 810 cc. The consistency was 1.46%. Two bacterial cellulose mixtures in water were prepared. The first had a consistency of 0.544% and was given 900 counts on the British disintegrator. The second had a consistency of 0.468 and disintegration was for 1800 counts.

Mixtures of pulp and bacterial cellulose were prepared which consisted of pulp at 0.5% consistency and of 1.0 or 3.0% bacterial cellulose. These mixtures were stirred with a Lightnin' mixer for times from 0.0 to 30 minutes, poured into a sheet mold, and allowed to drain. The drainage time was measured and found to be 5.6-6.6 seconds with no significant differences due to stirring time or bacterial cellulose content.

Handsheets were made incorporating various levels of bacterial cellulose in 1.55-g. sheets with and without rosin and alum size. For sizing, 2.0% rosin was stirred into the pulp bacterial cellulose mixtures for 5 minutes followed by 4% alum also stirred in for 5 minutes. Where necessary, the pH was adjusted to 4.5-5.0 with H_2SO_4. The sheets were pressed at 50 p.s.i. for 5 minutes and dried at 3.5 p.s.i. steam pressure for 7 minutes.

Table VIII summarizes the results of several physical tests made on the handsheets. Only the M.I.T. fold test result was "better," apparently due to the addition of 0.5 and 1.0% bacterial cellulose. Other physical properties clearly were not improved by bacterial cellulose as an additive, with or without size.

CONCLUSIONS AND FUTURE WORK

In part, the original stimulus for the investigation of cellulose formation by Acetobacter was to produce gram quantities of C-14 labeled cellulose, with high specific activity, for various research purposes. The results of early work clearly show that highest cellulose yields (30-40%) in a yeast extract-KH_2PO_4 culture medium were obtained at pH 6.5, 30°C., surface-to-volume ratio of 0.5, and after about 18 days' incubation. Later results with glucose-UL-C-14 in the medium indicated that the most efficient conversion of glucose to cellulose was at low concentrations of glucose and about 1.0% ethanol. It was found that ethanol-C-1-14, ethanol-C-2-14, aspartic acid-UL-C-14, and glutamic acid-UL-C-14 were incorporated in cellulose by Acetobacter at low levels in the presence of 0.1-10.0% glucose. However, alanine-UL-C-14 incorporation was significant (10-20%) at low glucose concentrations. Bacterial cellulose formation was inhibited when produced in the presence of 2-deoxy-2-fluoro-D-glucose, 2-deoxy-D-glucose, 3-deoxy-D-glucose, and D-mannose, and analysis by paper chromatography detected none of these sugars in the polymer.

In the formation of handsheets from bleached sulfite pulp, the addition of rehydrated bacterial cellulose at 0.5, 1.0, and 3.0%, with and without size, resulted in no changes in handsheet physical properties except for an apparent improvement in flexibility as measured by the M.I.T. fold test.

TABLE VIII

EFFECT OF BACTERIAL CELLULOSE ON HANDSHEET FORMATION

Type of Handsheet	Physical Test, average values						
	1	2	3	4	5	6	7
0.0% Bacterial cellulose, no size	44.2	4.1	10.8	64	53	16.5	80
0.5% Bacterial cellulose, no size	46.6	4.4	10.6	66	52	17.4	116
1.0% Bacterial cellulose, no size	46.8	4.5	10.4	62	51	15.9	114
*1.0% Bacterial cellulose, no size	46.6	4.5	10.4	62	51	14.6	72
3.0% Bacterial cellulose, no size	49.9	4.8	10.4	56	60	15.6	103
0.0% Bacterial cellulose, + size	46.9	4.4	10.7	60	57	15.0	59
0.5% Bacterial cellulose, + size	46.7	4.5	10.4	59	54	14.6	69
1.0% Bacterial cellulose, + size	46.2	4.5	10.3	59	54	15.6	89
*1.0% Bacterial cellulose, + size	45.4	4.4	10.3	59	53	14.4	66
3.0% Bacterial cellulose, + size	45.9	4.5	10.2	57	59	14.4	70

1. Basis weight, lb.
2. Thickness, mils
3. Apparent density.
4. Bursting strength (Mullen), pt. per 100 lb.
5. Elmendorf tear, g. per sheet.
6. Schopper tensile, lb. per inch.
7. M.I.T. fold.

* Bacterial cellulose mixture, 1800 counts British disintegrator.

In accordance with M. Johnson's memorandum to J. Swanson (June 8, 1970) IPC Project 2970 has been established. The title is "The Mechanism of Extracellular Polysaccharide Production by Acetobacter xylinum." This project redirects bacterial cellulose research to an investigation of nucleic acid involvement in the Acetobacter extracellular polysaccharide synthesizing system. Project 1829 should, therefore, be closed.