
AbSciCon
2019

1
00:00:00,790 --> 00:00:07,320
[Music]

2
00:00:11,170 --> 00:00:08,760
[Applause]

3
00:00:12,910 --> 00:00:11,180
in the my name is Trent Stubbs I'm a

4
00:00:14,740 --> 00:00:12,920
rising senior undergraduate student at

5
00:00:15,820 --> 00:00:14,750
Furman University and I've been working

6
00:00:17,890 --> 00:00:15,830
into the direction of dr. Greg

7
00:00:20,439 --> 00:00:17,900
Springsteen for the past two summers at

8
00:00:24,429 --> 00:00:20,449
a part of NASA and NSF funded Center for

9
00:00:26,620 --> 00:00:24,439
chemical evolution so I would like to

10
00:00:28,540 --> 00:00:26,630
start today by discussing the classic

11
00:00:30,490 --> 00:00:28,550
miller-urey experiment done in the 1950s

12
00:00:31,900 --> 00:00:30,500
it's really launched the field of

13
00:00:34,210 --> 00:00:31,910

prebiotic chemistry of course by

14
00:00:36,850 --> 00:00:34,220
demonstrating being able to transform

15
00:00:39,069 --> 00:00:36,860
these small organic materials into

16
00:00:42,040 --> 00:00:39,079
larger biologically relevant species

17
00:00:44,260 --> 00:00:42,050
like amino acids to other some more

18
00:00:46,510 --> 00:00:44,270
related experiments being formaldehyde

19
00:00:48,670 --> 00:00:46,520
condensation producing saccharides or

20
00:00:50,320 --> 00:00:48,680
HCN polymerization and hydrolysis

21
00:00:53,260 --> 00:00:50,330
generating a lot of precursors to

22
00:00:55,180 --> 00:00:53,270
nucleobases but as you can tell from the

23
00:00:57,700 --> 00:00:55,190
two HPLC chromatograms

24
00:00:59,500 --> 00:00:57,710
there was no thermodynamic end product

25
00:01:01,810 --> 00:00:59,510
to these processes and it's hard to

26
00:01:04,149 --> 00:01:01,820
imagine that biology could then take

27
00:01:06,910 --> 00:01:04,159
this system which is somewhat of an

28
00:01:08,890 --> 00:01:06,920
organic tar-like mess and be able to

29
00:01:12,070 --> 00:01:08,900
evolve from it and as some sort of

30
00:01:14,200 --> 00:01:12,080
established foundation so when we were

31
00:01:15,700 --> 00:01:14,210
designing our experiments to investigate

32
00:01:17,080 --> 00:01:15,710
the origins of metabolism we wanted

33
00:01:18,760 --> 00:01:17,090
something a little bit more robust and

34
00:01:20,860 --> 00:01:18,770
so we wanted to study something that

35
00:01:23,190 --> 00:01:20,870
exists in every living system ever and

36
00:01:26,020 --> 00:01:23,200
that is the circuit citric acid cycle

37
00:01:28,090 --> 00:01:26,030
these transformations now have many

38
00:01:30,000 --> 00:01:28,100

roles in modern biology three of which

39
00:01:32,920 --> 00:01:30,010
are to capture redox potential

40
00:01:34,810 --> 00:01:32,930
biosynthesize other relevant molecules

41
00:01:36,250 --> 00:01:34,820
and then fixate carbon in the form of

42
00:01:39,040 --> 00:01:36,260
the reductive citric acid cycle

43
00:01:41,260 --> 00:01:39,050
now these specific transformations to

44
00:01:43,240 --> 00:01:41,270
turn pyruvate an acetyl co a into these

45
00:01:45,010 --> 00:01:43,250
larger building block intermediates

46
00:01:47,649 --> 00:01:45,020
require eleven different enzymes and

47
00:01:52,030 --> 00:01:47,659
multiple Co factors including nad plus F

48
00:01:54,640 --> 00:01:52,040
ad and GDP but but of course we were

49
00:01:56,710 --> 00:01:54,650
just looking more for a some sort of

50
00:01:59,710 --> 00:01:56,720
remnant of these robusta geochemical

51
00:02:01,260 --> 00:01:59,720
pathways that existed within and many

52
00:02:04,210 --> 00:02:01,270
researchers before have tried to

53
00:02:05,770 --> 00:02:04,220
recapitulate these exact transformations

54
00:02:08,259 --> 00:02:05,780
that occur within the citric acid cycle

55
00:02:09,910 --> 00:02:08,269
and only two of which really proceeded

56
00:02:11,710 --> 00:02:09,920
in high yield that's the oxidative

57
00:02:14,800 --> 00:02:11,720
decarboxylation and the beta

58
00:02:17,110 --> 00:02:14,810
decarboxylation and and it makes very

59
00:02:19,119 --> 00:02:17,120
good sense for why these transformations

60
00:02:20,680 --> 00:02:19,129
don't occur the carboxylate is just

61
00:02:23,860 --> 00:02:20,690
simply not reactive enough

62
00:02:25,930 --> 00:02:23,870
the carbonyl carbon is not electrophilic

63
00:02:28,660 --> 00:02:25,940

enough for attack and then the carbon

64
00:02:31,330 --> 00:02:28,670
alpha 2 the carboxylate has a pKa of

65
00:02:33,280 --> 00:02:31,340
about 27 so it's just not something that

66
00:02:35,560 --> 00:02:33,290
is nucleophilic enough to be able to

67
00:02:37,510 --> 00:02:35,570
establish an appreciable equilibrium

68
00:02:41,890 --> 00:02:37,520
between the carboxylate and the either

69
00:02:44,230 --> 00:02:41,900
email or enolate to attack out of so we

70
00:02:46,000 --> 00:02:44,240
had an idea and now is to replace this

71
00:02:47,980 --> 00:02:46,010
carboxylate functional group with

72
00:02:49,510 --> 00:02:47,990
something more reactive and that more

73
00:02:51,610 --> 00:02:49,520
reactive group turns out to be something

74
00:02:54,100 --> 00:02:51,620
called an alpha keto acid and it's shown

75
00:02:57,220 --> 00:02:54,110
below on the bottom half of the screen

76
00:02:59,110 --> 00:02:57,230
and these alpha keto acids are much more

77
00:03:00,700 --> 00:02:59,120
electrophilic at that carbonyl carbon

78
00:03:02,830 --> 00:03:00,710
and are susceptible to attack and

79
00:03:05,860 --> 00:03:02,840
they're much more nucleophilic that

80
00:03:08,650 --> 00:03:05,870
alpha carbon next to the keto acid has a

81
00:03:10,030 --> 00:03:08,660
pKa of about 12 so now in an aqueous

82
00:03:13,060 --> 00:03:10,040
environment we can begin to

83
00:03:15,010 --> 00:03:13,070
deprotonating and start form the enol or

84
00:03:17,830 --> 00:03:15,020
enolate to attack out of as a good

85
00:03:20,230 --> 00:03:17,840
nucleophile and somewhat remarkably this

86
00:03:22,690 --> 00:03:20,240
alpha keto acid can then be transformed

87
00:03:24,910 --> 00:03:22,700
right back into the canonical carboxylic

88
00:03:27,790 --> 00:03:24,920

acid in near quantitative yield just

89
00:03:30,010 --> 00:03:27,800
through the oxidative decarboxylation so

90
00:03:31,780 --> 00:03:30,020
our idea was to take the citric acid

91
00:03:33,460 --> 00:03:31,790
cycle something robust in every

92
00:03:35,440 --> 00:03:33,470
biological system that exists and

93
00:03:37,060 --> 00:03:35,450
replaced one of those carboxylate

94
00:03:38,980 --> 00:03:37,070
functional groups with this more

95
00:03:40,960 --> 00:03:38,990
reactive alpha keto acid functional

96
00:03:43,750 --> 00:03:40,970
group and that's shown on the second

97
00:03:46,270 --> 00:03:43,760
line so for example Malley becomes now

98
00:03:48,520 --> 00:03:46,280
mal loyal for mate or fumarate becomes

99
00:03:51,729 --> 00:03:48,530
few more oil for me and you may even see

100
00:03:54,280 --> 00:03:51,739
that some keto acids exist now already

101
00:03:57,640 --> 00:03:54,290
in the citric acid cycle like alpha

102
00:03:59,199 --> 00:03:57,650
ketoglutarate and oxaloacetate and

103
00:04:01,180 --> 00:03:59,209
here's just an example of one reference

104
00:04:03,820 --> 00:04:01,190
in the literature right now utilizing

105
00:04:06,970 --> 00:04:03,830
the power of these alpha keto acids this

106
00:04:08,260 --> 00:04:06,980
was done by kim @l in the 70s where they

107
00:04:11,380 --> 00:04:08,270
were able to show the generation of

108
00:04:13,330 --> 00:04:11,390
citrate through a self condensation of

109
00:04:15,100 --> 00:04:13,340
oxaloacetate followed by beta

110
00:04:17,050 --> 00:04:15,110
decarboxylation and then oxidative

111
00:04:19,300 --> 00:04:17,060
decarboxylation to give reasonable

112
00:04:23,860 --> 00:04:19,310
yields in the aqueous environment at a

113
00:04:26,500 --> 00:04:23,870

pH of 5 in pH of 7 we wanted to test our

114
00:04:28,659 --> 00:04:26,510
prebiotic pathway and so we did that by

115
00:04:31,240 --> 00:04:28,669
reacting 200 millimolar pyruvate with

116
00:04:34,060 --> 00:04:31,250
three equivalents of glyoxylate and a pH

117
00:04:36,220 --> 00:04:34,070
7 buffer and that gave us these first

118
00:04:38,410 --> 00:04:36,230
these two NMR's shown and it was

119
00:04:40,600 --> 00:04:38,420
somewhat remarkable and that not only

120
00:04:43,150 --> 00:04:40,610
did we see the first aldol addition

121
00:04:45,940 --> 00:04:43,160
product that mal oil form eight species

122
00:04:48,400 --> 00:04:45,950
but we also saw the condensation product

123
00:04:49,900 --> 00:04:48,410
and then reduction product and then

124
00:04:52,090 --> 00:04:49,910
subsequent aldol addition and

125
00:04:53,500 --> 00:04:52,100
condensation product as well and this

126
00:04:56,050 --> 00:04:53,510
was really exciting for us and that's

127
00:04:58,690 --> 00:04:56,060
what the both NMR's are showing us a

128
00:05:01,360 --> 00:04:58,700
being starting from pyruvate and then be

129
00:05:03,430 --> 00:05:01,370
starting from alpha ketoglutarate where

130
00:05:06,070 --> 00:05:03,440
nearly everything we produced in

131
00:05:08,140 --> 00:05:06,080
solution was one of these citric acid

132
00:05:11,820 --> 00:05:08,150
cycle equivalence right just with the

133
00:05:14,410 --> 00:05:11,830
alpha keto acid functional group and

134
00:05:16,390 --> 00:05:14,420
then from there we wanted to transform

135
00:05:18,580 --> 00:05:16,400
this keto acid group back into the

136
00:05:19,900 --> 00:05:18,590
carboxylate functional group and we did

137
00:05:22,570 --> 00:05:19,910
that just through the oxidative

138
00:05:24,400 --> 00:05:22,580

decarboxylation ECAR box elation

139
00:05:25,810 --> 00:05:24,410
mechanism with five equivalents of

140
00:05:28,150 --> 00:05:25,820
hydrogen peroxide just at room

141
00:05:30,010 --> 00:05:28,160
temperature and in mere 15 minutes it

142
00:05:32,890 --> 00:05:30,020
gave us this beautiful spectra below

143
00:05:34,930 --> 00:05:32,900
where now everything in solution is now

144
00:05:36,790 --> 00:05:34,940
these citric acid cycle intermediates

145
00:05:39,400 --> 00:05:36,800
and this all stemmed from a single

146
00:05:41,410 --> 00:05:39,410
reaction pot a single system of just

147
00:05:43,810 --> 00:05:41,420
pyruvate and glyoxylate the smallest

148
00:05:47,740 --> 00:05:43,820
alpha keto acid and the second smallest

149
00:05:49,720 --> 00:05:47,750
alpha keto acid another way which we

150
00:05:53,170 --> 00:05:49,730
were able to visualize this reaction

151
00:05:55,060 --> 00:05:53,180
progression was by HPLC so two of the

152
00:05:57,730 --> 00:05:55,070
molecules in particular having a double

153
00:05:59,590 --> 00:05:57,740
bond really have a nice UV trace one

154
00:06:01,270 --> 00:05:59,600
being the few more oil formate labeled B

155
00:06:03,910 --> 00:06:01,280
and the other being a Cana torial

156
00:06:05,590 --> 00:06:03,920
formate labeled C so as we see that

157
00:06:08,260 --> 00:06:05,600
concentration of fumarole formate

158
00:06:10,450 --> 00:06:08,270
increase as we begin to go through the

159
00:06:12,910 --> 00:06:10,460
cycle we then see it reached its maximum

160
00:06:14,800 --> 00:06:12,920
and then start to fall as the

161
00:06:16,870 --> 00:06:14,810
concentration of the transit connot

162
00:06:19,150 --> 00:06:16,880
eight begins to increase so this was a

163
00:06:21,370 --> 00:06:19,160

beautiful pathway to really show that we

164
00:06:23,440 --> 00:06:21,380
were following this this cyclic system

165
00:06:25,810 --> 00:06:23,450
like that of the reductive reductive

166
00:06:28,870 --> 00:06:25,820
citric acid cycle and we believe that

167
00:06:30,700 --> 00:06:28,880
that transformation from be the fumarole

168
00:06:33,070 --> 00:06:30,710
format to the alpha ketoglutarate is the

169
00:06:35,050 --> 00:06:33,080
rate limiting step and it is presumed

170
00:06:37,360 --> 00:06:35,060
that that might proceed through a Kaunas

171
00:06:40,330 --> 00:06:37,370
ro type reduction of a hydride through

172
00:06:42,340 --> 00:06:40,340
the hydrate of glyoxylate additionally

173
00:06:45,280 --> 00:06:42,350
this helped us visualize that we were

174
00:06:47,480 --> 00:06:45,290
also producing some sis Akana Tate which

175
00:06:51,409 --> 00:06:47,490
is the biologically relevant ice

176
00:06:54,529 --> 00:06:51,419
of ikana tate and so this is the pathway

177
00:06:56,930 --> 00:06:54,539
in a nutshell the outer cycle being the

178
00:06:59,629 --> 00:06:56,940
modern reductive citric acid cycle and

179
00:07:01,670 --> 00:06:59,639
the inner cycle being our keto acid

180
00:07:03,980 --> 00:07:01,680
equivalent pathway starting from

181
00:07:06,529 --> 00:07:03,990
pyruvate and glyoxylate proceeding

182
00:07:08,330 --> 00:07:06,539
through the exact inner the exact

183
00:07:11,659 --> 00:07:08,340
equivalent of the intermediates in the

184
00:07:14,420 --> 00:07:11,669
citric acid cycle in a single reaction

185
00:07:17,450 --> 00:07:14,430
pot a single reaction system at a pH of

186
00:07:21,020 --> 00:07:17,460
seven and 50 degrees C and in only 21

187
00:07:23,120 --> 00:07:21,030
hours now you may see the one gap in

188
00:07:25,399 --> 00:07:23,130

between the Econo toil format in central

189
00:07:28,330 --> 00:07:25,409
formate that's one area in which we

190
00:07:31,730 --> 00:07:28,340
wanted to investigate in the future and

191
00:07:35,600 --> 00:07:31,740
we have now an idea for being able to

192
00:07:38,180 --> 00:07:35,610
transform that molecule the medial econo

193
00:07:40,879 --> 00:07:38,190
toll format we call it into the central

194
00:07:42,680 --> 00:07:40,889
forming if medial kana toriel form eight

195
00:07:45,559 --> 00:07:42,690
just hydrates in water it would be a

196
00:07:47,659 --> 00:07:45,569
conjugate addition and it would add not

197
00:07:49,490 --> 00:07:47,669
at the tertiary carbon which is give us

198
00:07:51,649 --> 00:07:49,500
the Satori form eight so we need to

199
00:07:53,749 --> 00:07:51,659
somewhat rearrange this keto acid

200
00:07:56,270 --> 00:07:53,759
functional group and we found that while

201
00:07:59,870 --> 00:07:56,280
taking an NMR of this species we would

202
00:08:02,330 --> 00:07:59,880
exchange protons with a deuterium in d2o

203
00:08:04,490 --> 00:08:02,340
at the beta carbon which would suggest

204
00:08:06,529 --> 00:08:04,500
we somehow activated that beta carbon

205
00:08:07,909 --> 00:08:06,539
and when you look at the structures and

206
00:08:09,920 --> 00:08:07,919
particularly the resonance structures of

207
00:08:12,140 --> 00:08:09,930
that species it makes sense why that

208
00:08:14,209 --> 00:08:12,150
carbon was activated it's now dumping

209
00:08:16,399 --> 00:08:14,219
into the carboxylate adjacent to it and

210
00:08:19,430 --> 00:08:16,409
then through the double bond into

211
00:08:21,499 --> 00:08:19,440
another carboxylate as well so in theory

212
00:08:23,600 --> 00:08:21,509
now we can add a second equivalent of

213
00:08:26,330 --> 00:08:23,610

glyoxylate ad at the beta position and

214
00:08:28,610 --> 00:08:26,340
then hydrate in retro out all that first

215
00:08:30,920 --> 00:08:28,620
glyoxylate addition giving us now

216
00:08:33,409 --> 00:08:30,930
terminal Akana toil for mate same

217
00:08:35,149 --> 00:08:33,419
molecule just having that car box re the

218
00:08:37,100 --> 00:08:35,159
keto acid a functional group in a

219
00:08:39,589 --> 00:08:37,110
different location this would then

220
00:08:41,180 --> 00:08:39,599
hydrate in the tertiary position we

221
00:08:43,430 --> 00:08:41,190
believe which would retro a little

222
00:08:46,430 --> 00:08:43,440
giving us two molecules of pyruvate and

223
00:08:48,139 --> 00:08:46,440
one molecule of carbon dioxide so if we

224
00:08:49,760 --> 00:08:48,149
can achieve this transformation now in

225
00:08:51,949 --> 00:08:49,770
the future that would have taken us from

226
00:08:55,930 --> 00:08:51,959
one pyruvate starting the cycle to two

227
00:08:57,680 --> 00:08:55,940
pyruvates and in excess of glyoxylate

228
00:09:00,439 --> 00:08:57,690
something else we wanted to demonstrate

229
00:09:01,639 --> 00:09:00,449
was how far can we take these alpha keto

230
00:09:04,219 --> 00:09:01,649
acids how

231
00:09:05,869 --> 00:09:04,229
can we utilize their reactiveness and

232
00:09:08,509 --> 00:09:05,879
and we wanted to generate some amino

233
00:09:10,129 --> 00:09:08,519
acids much like biology does with them

234
00:09:12,199 --> 00:09:10,139
now and so we did that through a

235
00:09:13,429 --> 00:09:12,209
transamination with the simplest amino

236
00:09:16,009 --> 00:09:13,439
acid glycine

237
00:09:18,559 --> 00:09:16,019
and a potassium aluminium salt at 80

238
00:09:21,710 --> 00:09:18,569

degrees C and a pH of 5 for about five

239
00:09:23,179 --> 00:09:21,720
hours and NMR a showing the generation

240
00:09:26,210 --> 00:09:23,189
of glutamate from alpha ketoglutarate

241
00:09:28,759 --> 00:09:26,220
and then B showing the generation of

242
00:09:30,619 --> 00:09:28,769
alanine from pyruvate in the presence of

243
00:09:33,229 --> 00:09:30,629
the glyoxylate and this potassium

244
00:09:37,249 --> 00:09:33,239
aluminium salt and then now somewhat

245
00:09:39,799 --> 00:09:37,259
interestingly is as well the glycine

246
00:09:41,689 --> 00:09:39,809
species then is also it's undergoing

247
00:09:44,509 --> 00:09:41,699
that transamination so it is producing

248
00:09:46,429 --> 00:09:44,519
glyoxylate which is the feed stock for

249
00:09:50,689 --> 00:09:46,439
our prebiotic cycle as shown on the

250
00:09:52,219 --> 00:09:50,699
previous slide and so just one more time

251
00:09:54,859 --> 00:09:52,229
just to kind of wrap up everything that

252
00:09:57,109 --> 00:09:54,869
was done on this project the inner cycle

253
00:09:59,089 --> 00:09:57,119
being our alpha keto acid equivalent in

254
00:10:03,079 --> 00:09:59,099
the outer cycle being the canonical

255
00:10:06,229 --> 00:10:03,089
citric acid cycle and a couple key

256
00:10:08,239 --> 00:10:06,239
points I want to emphasize or we're not

257
00:10:10,969 --> 00:10:08,249
suggesting that it was hydrogen peroxide

258
00:10:13,699 --> 00:10:10,979
or a potassium aluminium salt which

259
00:10:15,590 --> 00:10:13,709
under did these exact transformations

260
00:10:18,590 --> 00:10:15,600
but what we're trying to show is that

261
00:10:20,569 --> 00:10:18,600
going from these keto acids to the

262
00:10:23,119 --> 00:10:20,579
canonical carboxylates or two amino

263
00:10:25,309 --> 00:10:23,129

acids would have been really easy and

264
00:10:28,579 --> 00:10:25,319
really efficient and even advantageous

265
00:10:31,579 --> 00:10:28,589
for an evolving enzymatic system to to

266
00:10:34,309 --> 00:10:31,589
to use once it requires regulation

267
00:10:36,229 --> 00:10:34,319
because once we get the car nautical

268
00:10:38,840 --> 00:10:36,239
citric acid cycle intermediates as I

269
00:10:40,969 --> 00:10:38,850
showed on I think slide 2 they're dead

270
00:10:42,859 --> 00:10:40,979
ends and we can't undergo any further

271
00:10:45,559 --> 00:10:42,869
transformations unless we have that

272
00:10:49,489 --> 00:10:45,569
enzyme so we can imagine this as a

273
00:10:51,859 --> 00:10:49,499
possible prebiotic we plausible system

274
00:10:54,079 --> 00:10:51,869
for now in evolving enzyme to really get

275
00:10:57,829 --> 00:10:54,089
ahold of and grasp and be and to

276
00:10:59,539 --> 00:10:57,839
transform and evolve so with that I

277
00:11:00,829 --> 00:10:59,549
would just like to thank everyone who

278
00:11:02,419 --> 00:11:00,839
helped support this project Furman

279
00:11:04,639 --> 00:11:02,429
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280
00:11:07,339 --> 00:11:04,649
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281
00:11:09,499 --> 00:11:07,349
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282
00:11:11,449 --> 00:11:09,509
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283
00:11:13,489 --> 00:11:11,459
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284
00:11:14,230 --> 00:11:13,499
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285
00:11:16,690 --> 00:11:14,240
help

286
00:11:18,490 --> 00:11:16,700
operation on this product and lastly my

287
00:11:20,860 --> 00:11:18,500
lab members ELISA clay who's now at

288
00:11:22,990 --> 00:11:20,870

Stanford Rachel Cooke who will be giving

289
00:11:25,269 --> 00:11:23,000
a poster presentation tonight at seven

290
00:11:26,889 --> 00:11:25,279
and my p i-- and and mentor dr. Greg

291
00:11:28,180 --> 00:11:26,899
Springsteen so with that thank you so

292
00:11:36,010 --> 00:11:28,190
much for your attention I'd be happy to

293
00:11:58,640 --> 00:11:36,020
take any questions so we have time for

294
00:12:10,380 --> 00:12:06,690
yes right so we do see some oxalate in

295
00:12:12,360 --> 00:12:10,390
the NMR and particularly we believe

296
00:12:17,490 --> 00:12:12,370
there are a couple of competing pathways

297
00:12:19,920 --> 00:12:17,500
which might produce this the alpha

298
00:12:22,440 --> 00:12:19,930
ketoglutarate another one being through

299
00:12:22,950 --> 00:12:22,450
a second edition of glyoxylate but

300
00:12:24,360 --> 00:12:22,960
that's something we're still

301
00:12:25,500 --> 00:12:24,370
investigating and wanting to look down

302
00:12:27,060 --> 00:12:25,510
the road to really confirm that

303
00:13:00,270 --> 00:12:27,070
particularly with some isotopic labeling

304
00:13:01,860 --> 00:13:00,280
studies though I think it's a great

305
00:13:03,960 --> 00:13:01,870
question I think that's one of the the

306
00:13:06,570 --> 00:13:03,970
strongest points we have for this

307
00:13:09,630 --> 00:13:06,580
prebiotic cycle is that it runs by

308
00:13:11,010 --> 00:13:09,640
itself and therefore for some sort of

309
00:13:13,050 --> 00:13:11,020
living system there would be no

310
00:13:15,660 --> 00:13:13,060
regulation and there would be no control

311
00:13:18,120 --> 00:13:15,670
over what's occurring it can just go in

312
00:13:20,250 --> 00:13:18,130
water by itself pretty remarkable so

313
00:13:21,960 --> 00:13:20,260

then once the enzyme catalysis were

314
00:13:23,370 --> 00:13:21,970
available it could really kind of

315
00:13:26,780 --> 00:13:23,380
capture it and get a hold of what was

316
00:13:47,340 --> 00:13:26,790
going on that's exactly right

317
00:13:51,540 --> 00:13:49,410
so right now that that's just an

318
00:13:55,050 --> 00:13:51,550
oxidative decarboxylation mechanism and

319
00:13:57,960 --> 00:13:55,060
the currently the the cofactor to do

320
00:13:59,819 --> 00:13:57,970
that is TPP but we can do that with

321
00:14:01,650 --> 00:13:59,829
hydrogen peroxide which is just the

322
00:14:08,380 --> 00:14:01,660
byproduct of the photo oxidation of