PHARMACOLOGIC EFFECT OF DIPHTHERIA TOXIN AND DRUGS ON THE MECHANISM OF THE HEART BEAT IN CHICK EMBRYOES.

A study to correlate this mechanism with that for cardiac anaphylactic shock in chick embryoes.

by

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Submitted to the Department of Bacteriology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

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May 30, 1927

Acknowledgments

Grateful acknowledgment is hereby made to Dr. Noble F. Sherwood at whose suggestion this work was undertaken; and, without whose help and encouragement it would not have been possible for me to finish.

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INTRODUCTION

The demonstration by Sherwood (1) of cardiac anaphylaxes in chick embryoes ranging in age around 48 to 72 hours without apparent nervous tissue in the heart raises the question of whether anaphylaxes depends on nervous structure or the muscle tissue or both. Work in this laboratory and in others such as reported by Lillie (2) indicates no nervous tissue appears in the embryonic chick heart until the fifth or sixth day of incubation. Pottenger (3) from clinical studies on hypersensitiveness has analyzed the symptoms of hay fever and asthma and has considered these anaphylactic in nature and has explained these symptoms on the basis of parasympathetic stimulation. Stoland and Sherwood (4) have shown that Atropine Sulfate will inhibit the anaphylaxis phenomenon shown in smooth muscle of sensitized virgin guinea pigs. Auer (5) has shown that Atropine Sulfate frequently prevents the pulmonary phenomenon of anaphylaxes in the guinea pig. Gibbs (6) has worked with drugs selectively affecting the autonomic nervous system using the adult fowl. He found that Pilocarpine in the doses used had no

demonstrable effect on the vagus of the fowl. Atropine was found to paralyze the vagus of the fowl but normally he found no vagus tone present. He concluded that Physostigmin in doses of 0.5 mgm. to 1 mgm. per kilo greatly prolongs the effect of vagus stimulation but does not itself initiate inhibition of the fowl's heart. Gahringer (7) has shown in clinical anaphylaxis in pigeons that the symptoms are of profound weakness and dyspnea which suggest a cardiac manisfestation. The scope of the present paper is the investigation of any nervous mechanism which might be present in hearts of two to six day old chick embryoes using pharmacological methods.

METHODS USED

Eggs were obtained the same day as layed. In some instances the eggs were gathered every few hours in order that they would avoid being set on by the hens which might start development. They showed no superiority over those eggs which were gathered once a day and did not justify the added cost of obtaining them. The eggs were either put in the incubator at once or stored in the icebox at a temperature around 8 degrees C. They may be stored in the ice-box from one to two weeks and still not lose their ability to produce good embryoes. The 37.5 degree centigrade incubator in the bacteriology laboratory was used for incubation with excellent results. The first few days of incubation at this temperature, which is three degrees centigrade below that of the adult chicken, seems to be satisfactory. The eggs were turned every twenty-four hours.

Lewis (8) who has worked with the isolated heart of two and three-day chick embryoes points out that the heart rate is dependent upon a number of environmental conditions, such as temperature,

carbon dioxide tension, hydrogen-ion concentration, salt content, and other conditions of the medium, and upon mechanical stimulation, etc. The deeper the fluid bath over the heart, the faster the heart rate became when the cover of the bath was off; and, with the cover on the rate became still faster. These increases in rate he thought probably were due to an increase in CO_2 tension of the Locke-Lewis solution.

A number of experiments were performed at temperatures around 39 degrees centigrade. In this series of experiments an electrically heated and controlled water bath was used. The embryoes were never allowed to cool below 35 degrees centigrade during the time they were being transferred from the shell to the bath. After being transferred to the Locke-Lewis bath, they were tested as soon as possible to avoid irregularities which might show up later due to exposure outside the shell at this temperature. A number of experiments were made at room temperature which varied around 20 degrees centigrade to 25 degrees centigrade. The embryoes remained alive with regularly beating hearts up to 48 hours or longer. So far as could be observed they did not grow but only remained alive

at this temperature. The method of observation and recording of heart beats along with a time record was that used by Sherwood (1). On each embryo after a normal tracing was taken as a control, a measured quantity of the pharmacologic product was added to the 20 c.c. Locke-Lewis bath containing the embryo. The Diphtheria Toxin was obtained from the United States Public Health Service and was standardized as so many c.c. equal to an L-plus dose. This toxin was added with a pipette. The drugs were dissolved in Locke-Lewis solution and the so lution thus obtained was of known percentage composition. They were added (~~wither~~) with a graduated pipette or by drops, twenty drops equivalent to a c.c. The exact dosage of the drug added could be calculated. The time of starting a record on the drum or ending one was recorded as was the time at which any toxin or drug additions to the bath were made. The time was recorded at which any other manipulations, such as changing the bath of the embryo were made. By using these data and the second's tracing on the drum, any change of significance in the record of the heart beats could be calculated with reference to the time that it occurred. It was, of course, impossible to take a continuous tracing of the heart beats

throughout the experiment which in some cases lasted over a period of 24 hours. However, tracings were taken at significant times and observations were made but no tracings were taken. All tracings showing data of value of a normal or abnormal nature were preserved.

RESULTS

Table one gives the total number of eggs incubated and divides these according to eggs infertile or embryoes maldeveloped, embryoes used to gain technic, embryoes observed but untreated, and embryoes tested with a pharmacologic substance. This table furnishes a directory for the original data of table three.

RESULTS

Table I.

Eggs infertile or embryoes maldeveloped 174	Embryoes used to gain technic 78	Untreated	Embryoes tested 70	Total 331
2,3,4,5,6,7,8,9,10,11 12,13,15,16,17,18,21 23,26,28,30,36,39,40, 41,42,43,44,47,48,49, 50,51,53,54,55,56,57, 60,61,63,64,65,66,68, 69,70,71,72,73,74,76, 78,82,86,87,88,89,92, 93,94,95,99,104,106, 107,114,115,116,117, 121,125,126,154,155, 156,157,158,159,160, 161,162,163,164,165, 168,169,170,171,172, 173,178,179,180,181, 199,200,201,202,203, 194,195,196,197,198, 204,205,206,207,208, 209,223,224,225,226, 238,239,240,241,242, 243,249,250,260,263, 264,265,266,267,268, 269,270,271,272,284, 285,288,289,292,293, 294,295,296,297,298, 299,300,310,305,306, 302,308,309,310,311, 313,314,315,316,317, 318,319,320,321,322, 323,324,325,326,327, 328,329,330,331	1,14,20,25,32 33,34,35,52, 59,75,96,102, 105,109,110, 111,112,113, 118,119,120, 127,139,140, 141,142,143, 144,145,146, 147,148,182, 183,184,185, 190,191,192, 193,210,211, 212,213,214, 215,227,228, 234,235,236, 237,244,245, 246,247,248, 251,252,253, 254,255,256, 257,258,259, 273,274,275, 276,277,278, 279,280,281, 282,283	79 131 134 135 138 174 175 186 187	19,22,24,27, 29,31,37,38, 45,46,58,62, 67,77,80,81, 83,84,85,90, 91,97,98,100 101,103,108, 122,123,124, 128,129,130, 132,133,136, 137,149,150, 151,152,153, 166,167,176, 177,188,189, 216,217,218, 219,220,221, 222,229,230, 231,232,233, 261,262,286, 287,290,291, 302,303,304, 312	

Results from eggs subdivided as above with numerical number of each kind and the serial number of the egg below for reference to original data.

Table two concerns those embryoes tested. It gives the serial number of the embryo, the average temperature during the experiment, the incubator age of the embryo in hours, the pharmacologic agent used and its total amount and the result with certain added notations.

Table II.

Atropine with near Incubator Temperature

Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
286	36	60	Atropine S04	.050	−	No effect on heart rate
287	36	60	" "	.050	−	" " " " "
83	39	63	" "	.000943	−	No significant effect on heart rate
84	39	69	" "	.0013055	−	No effect on heart rate
85	39	70	" "	.0005	−	" " " " "
46	39	71	Physos-tigmin	.0005	+	Heart slowed and then became irregular
	39	71	Atropine S04	.0002	?	Along with change of bath and time made heart become irregular again.
37	39	73	Physos-tigmin	.0007	+	Marked decrease in heart rate
	39	73	Atropine S04	.00075	−?	Recovered to normal before Atropine S04 was added and Atropine showed no effect. Result not of much value either way because of previous addition of physostigmin.
90	39	88	Atropine S04	.00055	+	Slight increase in heart rate and then heart became irregular
62	39	90	Atropine S04	.00005	+	Heart became irregular

Table II. (cont.)

Serial Number	Av. Incub. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
Atropine with near Incubator Temperature						
91	39	91	Atropine SO4	.0015	−	No change in rate or rhythm
77	39	94	" "	.000055	?	Heart irregular to begin: no noticeable change
67	39	95	" "	.000005	+	Heart became irregular
80	39	99	" "	.000001	+	A decrease in heart. On further addition there was a great increase in heart rate followed by a decrease below normal. The heart became irregular.
81	39	101	" "	.000089	+	Heart rate decreased and then heart became irregular.
19	39	103	" "	.002	+	More rapid, then an irregular heart
22	39	118	Physostigmin Atropine SO4	.00015 .00055	+ ∓	Decrease in heart rate Increase in heart rate
58	39	118	Atropine SO4	.0002	?	Atropine sulfate failed to make a spontaneously irregular heart regular
24	39	122	" "	.0015	+	Initially it became faster and then irregular and slow
27	39	123	Physostigmin	.00015	+	Initial increase
	39	123	Atropine SO4	.0022	−?	Practically no change. Result not of much value either way because of previous addition of physos.
31	39	42? 146	" "	.0001	+?	Would start a stopped heart

TABLE II. (cont.)

Atropine with Room Temperature						
Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
176	24	51	Atropine SO4	.0055	–	No apparent drug effect
177	24	51	" "	.0055	–	" " " "
153	20	53	" "	.00055	–	" " " "
166	22	60	" "	.00055	+?	Very slight increase in rate
167	22	60	" "	.00055	–	No apparent drug effect
188	20.5	71	" "	.050	–	" " " "
189	22	71	" "	.010	–	" " " "
						Failed to get anaphylactic shock on this embryo afterwards
216	23	72	" "	.010	–	No apparent drug effect
217	23	72	" "	.050	–	" " " "
218	25.5	72	" "	.030	–	" " " "
219	25	72	" "	.020	–	" " " "
220	24	84	" "	.050	–	" " " "

Table II. (cont.)

Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
Atropine with Room Temperature						
221	24	84	Atropine SO4	.050	−	Massive dose gave slight irregularity
222	24.5	84	" "	.050	−	Heart irregular with recovery
229	25.5	87	" "	.050	−	Heart irregular with recovery
230	25	87	" "	.050	−	Heart became irregular
	37	87	" "	—	−	Irregularity accentuated
Pilocarpine with near Incubator Temperature						
312	36	48	Pilocarpine	.050	−	No effect on heart rate
302	36	71	Pilocarpine	.050	+?	Slight irregularity during first three minutes after adding drug but heart was perfectly regular after that. Absorption generally requires two or more minutes and raises the question of physical stimulation in this case.
303	36	71	Pilocarpine	.050	−	No effect on heart rate
304	37	71	Pilocarpine	.050	+	Heart became irregular

Table II. (cont.)

Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
Pilocarpine with near Incubator Temperature (Philocarpine S04)						
290	36	73	Pilo-carpine	.050	+	No effect on heart rate
291	37	73	Pilo-carpine	.050	−	Vagus-like effect with slowing of heart. No irregularity
261	36	85	Pilo-carpine	.050 .050	−	Slowed heart (vagus-like effect) and then heart became irregular. Changed bath and on second addition of drug heart was slowed again
Pilocarpine with Room Temperature						
233	27	82	Pilo-carpine	.050	−	Heart became irregular
	37	82	Pilo-carpine	No more Added	−	Heart remained irregular
231	25.5	87	Pilo-carpine	.050	−	Heart became irregular
	37	87	Pilo-carpine	No more Added	−	Irregularity accentuated
232	26	87	Pilo-carpine	.050	−	Heart became irregular

Table II (cont.)

Physostigmin SO4, with near incubator temperature						
Serial Number	Av. Incub. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
46	39	71	Physostigmin	.0005	+	Heart slowed and then became irregular
37	39	73	Physostigmin	.0007	+	Marked decrease in heart rate
62	39	90	Atropine SO4	.00005	+	Heart became irregular
	39	90	Physostigmin	.00015	?	Heart remained irregular
22	39	118	Physostigmin	.00015	+	Decrease in heart rate
27	39	123	Physostigmin	.00015	+?	Initial increase. Transposed action?
	39	123	Atropine SO4	.0022	+?	Practically no change
	39	123	Physosmin	.002	+	Decrease in rate and then irregular
29	39	128	Physosmin	.002	+	Very slight decrease in rate and then heart became irregular
31	39	146 (42?)	Atropine SO4	.0001	+?	Would start a stopped heart
	39	146 (42?)	Physostigmin	.0001	+?	Would cause heart to stop

Table II. (Cont.)

Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
Adrenaline CL2 containing Chlorotone with near incubator temperature						
45	39	70	Adrenaline containing Chlorotone	.00025	?	A stopped heart was started
38	39	77	Adrenaline containing Chlorotone	.00075	?	Slight increase in rate and then heart became irregular
Strychnine S04 with near incubator temperature						
100	39	66	Strychnine S04	.010	+	Marked decrease in heart rate
101	39	77	Strychnine S04	.00505	+	Regular throughout. Decrease in rate followed by an increase over normal
103	39	83	Strychnine S04	.0075	+	Marked decrease in heart rate
97	39	210	Strychnine S04	.0005	?	No result obtained on heart. Embryo showed movement either passive or active
98	39	210		.0025	?	Heart not beating at start. No body movements were observed.

Table II. (cont.)

Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Drugs	Presence of Effect believed due to Drugs	Result
Caffeine Citrate						
108	39	53	Caffeine Citrate	.00555	−	No change
Tincture of Digitalis						
122	39	43	Tr. Digitalis	(5.5cc)	?	Heart stopped. Probably due to alcohol in the tincture
Ethyl Alcohol						
123	39	44	C_2H_5OH	(5.5cc)	+	Heart stopped. Probably direct effect on protoplasm.
Ether						
124	39	47	Ether	(0.5cc)	+	Heart slowed. Probably direct effect on protoplasm
Chloroform						
128	39	73	Chloroform	(1.25 cc)	+	Slowed the heart. Later it became irregular. Effect on protoplasm probably
Barium Chloride						
261	36	85	Pilocarpine	.050 .050	+	Slowed heart (vagus-like action) and then heart became regular. Changed both and on second addition of drug heart slowed again

Table II. (cont.)

Serial Number	Av. Temp. in C. of Exp.	Incub. Age in Hours	Toxin or Drug Added	Dosage in Grams	Presence of Effect believed due to Drugs	Result
Barium Chloride						
	36	85	Barium CL2	.050	–	Stopped heart permanently sometime within an hour. Probably direct effect on proto.
Ergotoxin						
262	26	85	Ergotoxin	.0013	?	Heart became irregular but was slightly irregular before
Diptheria Toxin			Toxin added	Lt doses	Lethal Effect	
129	22	50	Diptheria	1	–	No lethal effect
130	22	50	Diptheria	1	?	Heart became arrhythmic but didn't stop
132	23	79	Diptheria	1	–	No lethal effect
133	23	79	Diptheria	1	–	No lethal effect
136	21	80	Diptheria	1	–	No lethal effect
137	21	80	Diptheria	1	–	No lethal effect
149	22	82	Diptheria	1	–	No lethal effect
150	22	82	Diptheria	1	–	No lethal effect
151	22	82	Diptheria	1	–	No lethal effect
152	22	82	Diptheria	1	–	No immediate lethal effect. Embryo was separated from yolk and eventually died.

In table two the embryoes have been divided according to the pharmacologic agent used and subdivided as to whether the experiment was carried out at near incubation temperature or at room temperature. The embryoes are arranged in the order of increasing age. Of the drugs used, the results obtained with Atropine Sulfate, Pilocarpine Sulfate, and Physostigmin Sulfate are most important since these drugs selectively affect first the autonomic nervous system.

A perusal of table two shows that Atropine Sulfate in doses of 0.0009 grams to 0.05 grams gave an unquestionable negative response in five embryoes (#286, *287, *83, *84, *85) ranging in incubator age from 60 to 70 hours. An apparent negative response occurred in an embryo (#91) of 91 hours incubator age with 0.0015 grams of Atropine Sulfate and a questionable negative response in embryoes of 73 hours (#37) and 123 hours (#27). With the former, 0.00075 grams of Atropine Sulfate were used and with the latter 0.0022 grams. Apparent positive responses were obtained with eight embryoes (#90, *62, *67, *80, *81, *19, *24, *22) ranging in incubator age from 90 to 122 hours using Atropine Sulfate in doses ranging from

0.000001 grams to 0.002 grams at near incubator temperature. A questionable positive response was obtained in an embryo (#31) of 146 hours incubator age using 0.0001 gram of Atropine Sulfate.

It was found, as shown in table two, with Atropine Sulfate at room temperature that the eleven embryoes (#176, *177, *153, *167, *188, *189, *216, *217, *218, *219, *220) which gave negative responses, using doses of 0.00055 grams to 0.05 grams, ranged in incubator age from 51 to 84 hours. Four embryoes (#221, *222, *229, *230) which gave apparently positive responses using 0.05 grams of the drug ranged in incubator age from 84 to 87 hours. A questionable positive response was obtained in an embryo (#166) of 60 hours with 0.00055 grams of Atropine Sulfate.

It was found with Pilocarpine Sulfate at near incubator temperature that the three embryoes (#303, *290, *312) which reacted negatively using 0.05 grams of the drug ranged in incubator age from 48 to 73 hours. The three (#304, *291, *261) which apparently reacted positively using 0.05 grams of Pilocarpine Sulfate were of 71, 73, and 85 hours incubator age respectively. A questionable positive reaction was obtained in an embryo (#302) of 71 hours

incubator age using 0.06 grams of the drug.

As shown in table two with Pilocarpine Sulfate at room temperature apparent positive reactions were obtained with three embryoes (#233,#231,#232) ranging in incubator age from 82 to 87 hours using 0.05 grams of the drug.

As shown in table two, Physostigmin Sulfate was used at near incubator temperature. Apparently positive reactions were obtained in five embryoes (#46, #37, #22, #27, #29) ranging in incubator age from 71 to 128 hours using 0.00005 grams to 0.002 grams of the drug. A questionable positive reaction was obtained with one embryo (#31) of 146 hours using 0.0001 grams of the drug. In the same table it may be observed that Strychnine Sulfate in doses of 0.005 grams to 0.01 grams affected the heart rate in three embryoes ranging in age from 66 to 83 hours. It probably affected the cardiac muscle directly. Results obtained with adrenaline containing Chlorotone, Coffeine Citrate, Tincture of Digitalis, Ethyl Alcohol, Chloroform,

Ergotoxin, and BariumChloride may be observed in table two. They are too meager to be of value except in that they point out that the protoplasm of the heart muscle was affected directly in embryoes younger than 71 hours. The cardiac mechanism of embryoes younger than 71 hours does not seem to be affected by drugs such as Atropine, Physostigmin, and Pilocarpine, which have a specific action on the parasympathetic division of the autonomic nervous system. The recent work of Johnstone (9) still leaves the exact mechanism of the heart beat of chick embryoes in doubt. He tried ligatures around the atrio-ventricular juncture in the two, three and four-day chick hearts and produced heart block. He observed that the rate of the atrium, ventricle, and bulbus arteriosus varied both when a ligature was placed on the heart and when they were cut apart and observed in Locke-Lewis solution.

Probably the action of the Tincture of Digitalis was due to the alcohol present in the tincture. The action of Adrenaline may have been due to the presence of the chlorotone in it. Caffeine Citrate may have acted directly on the cardiac muscle of

the 53 hour old chick embryo. As shown in table two no lethal effest was obtained with Diphtheria Toxin in the concentration used.

Table three contains the original data collected:

Table III.

TABLE CONTAINING ALL ORIGINAL DATA

July 15, 1926 Put 4 dozen eggs at 1:00 P.M.

July 19, 1926

#1. 3:45 P.M. Good embryo but ruptured blood vessels in taking out.

#2. 3:55 P. M. Egg not fertile

#3. 4:00 P. M. Egg not fertile

#4. 4:02 P.M. Egg not fertile

#5. 4:05 P. M. Egg not fertile

#6. 4:08 P. M. Just blood islands no embryo.

#7. 4:10 P. M. Infertile

#8. 4:14 P. M. Blood islands and "monster".

#9. 4:17 P. M. Infertile

#10. 4:23 P. M. Infertile

#12. 4:50 P. M. Heart stopped.

#13. 4:55 P. M. Infertile

#14. 5:00 P. M. Embryo O.K. but so large it was difficult to handle. When with yolk could not see heart. Isolated from yolk but hemorrhage caused loss of blood and when view of heart was finally obtained it was not beating.

#15. Blood islands only

#16. Infertile

#17. Infertile

#18. A few blood islands

#19 ~~(or 160.)~~ Put July 15, 1926 at 1 P. M.
8:45 P. M. Took out good embryo. In 20 cc of L. L. Blood vessels in perfect shape.
8:48 P. M. First tracing
8:51 P. M. Added ten drops of Atropine 0.1%.
8:52 P. M. Second tracing started
8:56 P. M. Third tracing started
9:00 P. M. Fourth tracing started
9:03 P. M. Fifth tracing started
9:06 P. M. Sixth tracing started
9:08 P. M. Added 10 drops of Atropine 0.1%.
9:22 P. M. Added 10 drops of Atropine 0.1%. Heart still beating rather irregularly.
9:27 P. M. Added 10 drops of Atropine 0.1%. Heart now beating only at intervals and slowly.
9:33 P. M. Added 10 drops of Physostigmin 0.1%. Heart continued to beat irregularly.

Some embryoes develop arrithymia soon after being opened; some do not. This embryo showed a marked change soon after the Atropine was added. It might be due to the Atropine or it might have occurred anyway. However technic was well enough developed so that there was very little physical irritation in placing the embryo in its bath and it was beating in perfect rhythm before the Atropine was added. Moreover the time after it was taken out of the shell to the time the Atropine was added was only six minutes and as a rule irregularity does not spontaneously develop until much after that time: a half an hour or more. It would seem that this embryo had a nervous system to respond to Atropine.

#20. 10:00 P. M. Good embryo but was not successful in getting it out into bath.

Today in addition to running above embryoes I prepared two bottles of Locke-Lewis solution, made up 100 cc of 0.1% solution of the following: Atropine, Physostigmin, and Pilocarpine, got shellac and coal oil, got 4 dozen eggs, and put 9 eggs into incubator at 8:00 P. M.

July 20, 1926

#21. 9:20 A. M. Blood islands and a "monster" embryo.

#22. 10:10 A. M. Taken out into 20 cc of L. L. Then put in 2cc of L. L.
10:45 A. M. Tracing 1 - normal - 80 beats to a minute.
10:50 A. M. Added 1 drop of 0.1% Physostigmin.
10:51 A. M. Tracing #2.
10:55 A. M. Added 1 drop of 0.1% Physostigmin.
10:58 A. M. Tracing #3/
10:59 A. M. ½ tracing #4 - 70 beats to a minute.
11:02 A. M. Heart stopped.
11:05 A. M. Heart beating again
11:10 A. M. Added 1 drop of 0.1% Physostigmin.
11:11 A. M. Heart stopped.
11:14 A. M. Tracing - 75 beats to a minute.
11:20 A. M. Added 1 drop of Atropine. Heart began to beat - may have been due to irritation since drug was added right over heart.
11:23 A. M. Tracing #7 - 75 beats to a minute(½)
11:25 A. M. Added 10 drops of Atropine at side of dish.
11:26 A. M. Heart began to beat. Might have been due to Atropine or because it was going to beat anyhow.
11:27 A. M. Second part of tracing #7 - 90 beats to a minute.

This embryo was perfectly all right and regular for 40 minutes until the Physostigmin was added. It then became slow at times and stopped at intervals. When

they become irregular spontaneously the time when they do beat is much faster than the normal rate and appears to be a sort of compensatory reaction so that the total number of beats over a long period is nearly normal. However, in this embryo when the heart did beat it was slow in indicating the effect of the physostigmin in activating the vagus *if present.*

#23. 2:00 P. M. Took out of shell. Well developed embryo. When a view of the heart was obtained it was not beating. It was observed for ten minutes but it did not beat again.

#24. 2:30 P. M. Took out good embryo into about 5 cc of L. L. Heart beating well.
2:40 P. M. 1st tracing - 125 beats to a minute.
2:43 P. M. Added 1 drop of 1% Atropin.
2:48 P. M. Tracing #2 - 125 beats to a minute.
2:51 P. M. Added 1 drop of 1% Atropin.
2:55 P. M. Tracing #3 - 140 beats to a minute.
3:00 P. M. Added 1 drop of 1% Atropin.
3:03 P. M. Added 1 drop of 1% Atropin.
3:06 P. M. Added 1 drop of 1% Atropin.
3:10 P. M. Added 1 drop of 1% Atropin.
3:10 P. M. ½ tracing #4.
3:13 P. M. Tracing #5.
3:18 P. M. Added 20 drops of .1% Atropine.
3:20 P. M. Tracing #6: Is Atropin acting as a general protoplasmic poison on the heart muscle to cause it to stop or would the embryo have acted that way anyhow? It might be there is no vagus function and that the Atropin is cutting on the heart tissue itself.

#25. 4:10 P. M. Ruined in removing from shell. Good embryo.

#26. 4:15 P. M. Blood islands and "monster" embryo.

#27 or #163. 4:20 P. M. Took out good embryo. Yolk ran off but all membranes present. In 4cc of

L. L.
4:39 P. M. Tracing #1 - 90 beats to a minute.
4:42 P. M. Added 1 drop of 0.1% Physostigmin.
4:44 P. M. ½ tracing #2 - 115 beats to a minute.
4:47 P. M. Added 1 drop of 0.1% Physostigmin.
4:48 P. M. Last ½ of tracing #2.
4:50 P. M. Added 1 drop of 0.1% Physostigmin.
4:51 P. M. Tracing #3 - 120 beats to a minute.
4:55 P. M. 2 drops of 0.1% Atropine added.
4:56 P. M. ½ of tracing #4.
4:52 P. M. Added 2 drops Atropine.
5:00 P. M. Last ½ of tracing #4 - 115 beats to a minute.
5:04 P. M. Added 10 drops of Physostigmin .1%.
5:05 P. M. ½ tracing #5.
5:07 P. M. Added 10 drops of Physostigmin .1%.
5:12 P. M. " 10 " " " .1%.
5:13 P. M. Tracing #6 - ½ of - 80 beats to a minute.
5:14½ P. M. Added 10 drops Physostigmin, .1%.
5:15 P. M. Last ½ tracing #6.
5:17 P. M. Tracing #7.

#29 or 164. 8:50 P. M. Good embryo taken out in 4 cc of L. L.
9:26 P. M. Tracing #1 - 110 beats to a minute.
9:29 P. M. 2 drops of Physostigmin .1%.
9:30 P. M. ½ of tracing #2.
9:32 P. M. Added 8 drops of .1% Physostigmin.
9:34 P. M. Added 10 drops of .1% Physostigmin.
9:42 P. M. Added 20 drops of .1% Physostigmin.
9:46 P. M. Added 20 drops of .1% Physostigmin.
9:48 P. M. Heart had been perfectly regular but stopped for a minute: when it resumed beating it was at a slow rate: not fast as in spontaneous irregularity.
9:51 P. M. Tracing #3.
9:54 P. M. Tracing #4 - 100 beats to a minute.
9:58 P. M. Tracing #5 - Started on purpose when heart had stopped to show how it would beat after it got over the stopped period. Note that it assumed the normal rate when it did beat.

July 21, 1926

#30. 2:20 P. M. Blood islands and "monster" embryo.

#31 (~~or #165~~) 2:30 P. M. (42 hours old?)
2:52 P. M. Started tracing but heart had just (~~had and~~) stopped.
2:53 P. M. Added 2 drops of Atropin and heart began to beat again.
2:54 P. M. Added 4 drops of Physostigmin and heart stopped.
2:54½ P. M. Added 1 drop of L. L. and got no effect. Then added 2 drops of Atropine and heart began to beat.

Experiments suggestive but perhaps heart would have done the same thing without the drugs.

#32. 6:55 P. M. Good embryo but blood vessels were so extensive that it could not be gotten out successfully. 150 hour chick embryo.

#33. 7:10 P. M. Good embryo but blood vessels were so extensive that it could not be gotten out successfully. 150 hour chick embryo. Conclude that it is useless to try and use 150 hour chick embryoes. Will open up several of remaining eggs of this set from day to day and note developement. Might be possible later on to test them.

July 22, 1926

#34. 7:10 P. M. Took out embryo of 180 hours under L. L. A blood vessel broke but the blood was observed to clot in 4 minutes, 20 seconds. The heart was irregular and unsuited for experimenting purposes.

#35. 7:40 P. M. Took out 180 hour embryo under L. L. Movement of rbc through blood vessels could be observed but the heart was so buried beneath membranes it could not be observed. In the previous embryo (#34) the heart could not directly be seen but one of the large blood vessels was seen to move, evidently being pulsated by the heart.

#36. 8:20 P. M. Taken out of shell. Put in incubator at 8:00 P. M., July 19, 1926. Embryo then 72 hours old. In 10 cc of L. L.
8:35 P. M. Was irregular and could not be used.

#37 (~~or #166.~~) 8:50 P. M. Took out 73 hour embryo, in 10 cc of L. L.
8:58 P. M. Took tracing #1 for normal - 95 beats to the minute.
9:02 P. M. Add 4 drops of Physostigmin .1%.
9:05 P. M. Tracing #2.
9:09 P. M. Add 10 drops of .1% Physostigmin.
9:10 P. M. Made bath up to 20 cc.
9:13 P. M. Tracing #3.
9:16 P. M. Tracing #4 - 50 beats per minute.
9:25 P. M. First part tracing #5 - 80 beats per minute.
9:26 P. M. Added 10 drops .1% Atropin.
9:31 P. M. Last part tracing #5 - 85 beats per minute.
9:36 P. M. Added 5 drops of .1% Atropin.
9:42 P. M. Tracing #6 - 80 beats per minute.

July 23, 1926

#38 (~~or #167~~) 10:30 A. M. Took out embryo. Into 20 cc of L. L. Blood clotting mechanism present at this time.
10:57 A. M. Tracing - 55 beats per minute.
11:00 A. M. Added 5 drops of 1-1,000 Adrenalin.
11:05 A. M. " 10 " " " "
11:06 A. M. Tracing #2 - 65 beats per minute.
11:16 A. M. Changed bath, rinsing once.
11:10 A. M. Tracing #3.
11:40 A. M. Changed bath.
1:08 P. M. Tracing #4 - shows that when heart did beat it was regular. Show typical pauses. Evidently adrenalin had an effect in tracing #3.
1:12 P. M. Added 2 drops of adrenalin.
1:17 P. M. Added 2 drops of adrenalin.

#39. 1:30 P. M. Infertile egg.

#40. 1:35 P. M. Infertile egg.

#41. 1:40 P. M. Infertile egg.

#42. 1:45 P. M. Infertile egg.

#43. 1:50 P. M. Infertile egg.

#44. 2:00 P. M. Well developed embryo but heart was not beating when it was exposed.

July 24, 1926 - Saturady - Put in 9 eggs at 8:00 P. M.

July 26, 1926

#45. Put in July 15, 1926. Can not see heart but can observe blood flow.
8:15 A. M. Taken out.
8:40 A. M. Blood not circulating.
8:42 A. M. Added 5 drops of 1-1000 epinephrin to 20 cc bath.
8:50 A. M. Blood had not resumed circulating.

#46, ~~(or #169)~~ Put in July 23, 1926
9:00 A. M. Took out embryo - into 20 cc of L. L.
9:16 A. M. Tracing #1 - 105 beats per minute.
9:20 A. M. Added 10 drops of Physosligmin .1%.
9:20½ A. M. Tracing #2.
9:22½ A. M. Tracing #3 - 70 beats per minute.
9:28 A. M. Tracing #4.
9:50 A. M. First half of tracing #5.
9:52 A. M. Added 2 drops of .1% Atropin.
9:59 A. M. Rest of tracing #5.
10:00 A. M. Tracing #6. Noted by observation in between tracings that pauses of the heart were not as long. Also shown by tracing. Also shown on tracing that heart was beating fastor. Perhaps the Atropine was taking away the effect of the Physostigmin on the vagus.
10:10 A. M. Changed bath rinsing once 20 cc L. L.
10:30 A. M. First part of tracing #7. Period when heart was beating during this time it would stop at intervals as observed at times not during the tracing. 85 beats per minute.
10:35 A. M. One drop of Atropine added.
10:44 A. M. One drop of Atropine added.
10:52 A. M. It is noted that when the ventricle stops the auricles do too.
11:05 A. M. Added 5 drops Fr. of digitalis.
11:10 A. M. " " " " " "
11:20 A. M. " 20 " " " "

11:36 A. M. Heart block has been observed. The auricles would beat as much as 10 to 20 times and then the ventricle for a while with the same rhythm as the auricle. The ventricle did not go into fibullation, that is when it did beat its beats coincided with the beats of the auricle. The fact that alcohol is present in this tincture of digitalis should be taken into account in drawing conclusions.

#47. 2:05 P. M. Infertile.

#48. 2:20 P. M. Infertile.

#49. 2:30 P. M. Infertile.

#50. 2:35 P. M. Infertile.

#51. Put in July 23, 1926.
2:45 P. M. Took out embryo.
3:10 P. M. Good embryo but back was up so the heart could not be seen.

#52. Put in July 23, 1926.
3:25 P. M. Embryo O.K. but yolk broken in getting out.

Put 12 eggs mark 26 at 4:00 P. M.

July 26, 1926. Rearranged apparatus and read article in library.

#53. Put in July 23, 1926. 3:45 P. M. Infertile.

#54. Put in July 23, 1926. 3:55 P. M. Took out embryo. It's back was upward so that heart could not be observed.

#55. 4:14 P. M. Infertile.

July 28, 1926.

#56. Put in July 23. 7:40 A. M. Infertile.

#57. Put in July 23, 1926.
7:50 A. M. Took out embryo

8:15 A. M. Irregular heart so was discarded.

#58 ~~(or #169)~~ Put in July 23, 1926.
8:24 A. M. Taken out into 10 cc of L. L. (with 20 cc could not see heart)
8:50 A. M. Tracing #1 showing irregularity. If heart is inhibited by vagus then by adding Atropine this inhibition should be removed.
8:55 A. M. Added 4 drops of 1% Atropine.
9:12 A. M. Irregularity not removed. Would indicate that heart does not stop after physical irritation by the mechanism of vagus inhibition but because of direct effect on heart muscle. The irregularity might be due to copper salts or an unbalance of ions in Locke-Lewis solution.

Spent time trying to find some kind of dish to line inside of copper heater. A 250cc beaker has the right diameter but is too tall. Finally used two small beakers, one inside the other. This is not very satisfactory but illiminates chances of copper salts getting to the embryoes.

#59. Put in July 23, 1926.
11:30 A. M. Yolk broken and it became arrythmic.

#60. Put in July 23, 1926.
2:00 P. M. Infertile.

#61. Put in July 24, 1926.
2:05 P. M. Infertile.

#62, ~~(or #170)~~ Put in July 24, 1923. (90 hours old)
2:10 P. M. taken out in 20 cc of L. L. Good condition
2:26 P. M. Tracing #1 - perfect rhythm - 65 beats per minute.
2:31 P. M. Added 1 drop of.1% Atropine.
2:35 P. M. Observed very fast heart
2:36 P. M. Tracing #2

2:40 P. M. Changed bath rinsing 3 times
3:05 P. M. Changed bath rinsing once. Just before this change the heart was beating like tracing #2.
3:19 P. M. Tracing #3 - showing continued irregularity.
3:22 P. M. Added 3 drops of Physostigmin .1%.
3:33 P. M. Heart still irregular. If irregularity was due to action of Atropin on vagus ending then Physostigmin instead of toxic action on heart muscle (in right amount) should remove this vagus effect.
3:40 P. M. Tracing #4.
3:44 P. M. Tracing #5. Has periods of stoppage. Then it will beat for a time in perfect rhythm.

#63. Put in July 24, 1926.
4:50 P. M. Blood islands only.

#64. Put in July 24, 1926.
4:55 P. M. Blood islands not connected with slightly developed embryo.

#65. Put in July 24, 1926.
5:00 P. M. Same as #64. Started to develop but ceased.

#66. Put in July 24, 1926.
5:05 P. M. Took out embryo. In 20 cc of L. L.
5:17 P. M. Heart irregular - discarded.

#61 ~~(or #171)~~. Put in July 24, 1926. (95 hours)
6:40 P. M. Embryo taken out in 20 cc L. L.
6:52 P. M. Tracing #1.
6:55 P. M. Added 1 drop of 0.01% (dil) Atropine.
6:59 P. M. Observed heart to sudden beat-very fast. It had a perfect, slow rhythm before this. Surely must have been the effect of the atropine.
7:00 P. M. Tracing #2.
7:04 to 7:10 P. M. Rinsed three times and changed bath.
7:20 P. M. Tracing #3 - started just at the beginning of a rest period.
7:32 P. M. Added 2 drops of .1% Physostigmin.
7:44½ P. M. Tracing #4.
7:47 P. M. Tracing #5.

#68. Put in July 24, 1926
8:27 P. M. Infertile.

#69. Put in July 24, 1926.
8:35 P. M. Taken out in 20 cc L. L.
8:55 P. M. Irregular

July 29, 1926. Did not work.

July 30, 1926.

7:40 A.M. tools lost
Moved

#70. 11:20 A. M. Infertile - put in July 15, 1926.

#71. 11:30 A. M. Put in July 15, 1926. Blood islands only.

#72. 11:35 A. M. Put in July 15, 1926. Blood islands only. It is noted that if blood islands exist then the yolk becomes darker in color and liquifies. The white decreases markedly. Even blood islands alone start the yolk of the egg through its cycle of changes.

#73. 11:40 A. M. Put in July 15, 1926. Infertile.

#74. 11:42 A. M. Put in July 15, 1926. Infertile.

#75. 1:05 P. M. Took out - good embryo- yolk broken.

#76, 1:20 P. M. Infertile. Put in July 26, 1926.

#77 ~~(or #178)~~ Put in July 26, 1926. (94 hour embryo)
1:25 P. M. Took out good embryo in 20 cc of L. L.
1:58 P. M. Tracing #1 - heart slightly irregular. Use for control showing non-drug arrhythmia
2:04 P. M. Tracing #2.
2:15 P. M. Tracing #3.
2:30 P. M. Tracing #4.
2:47 P. M. Tracing #5.
3:00 P. M. Tracing #6.
3:04 P. M. Added 1 drop of .1% Atropine.
3:10 P. M. Added 1 drop of .1% Atropine.
3:21 P. M. Tracing #7 - if anything the heart is more regular. The Atropin did not have the expected effect of causing pauses followed by rapid beating periods for this dosage of Atropine. Here seems to be a case where either:

1. Cell permanability was too high for absorption from solution
2. No nervous mechanism present
3. This heart muscle was not susceptable to this conc. of the drug.

#78. Put in July 26, 1926
3:37 P. M. Blood islands only.

#79 ~~(or 185)~~ Put in July 26, 1926 (96 hour embryo)
3:45 P. M. Took out in 20 cc or L. L.
4:21 P. M. Tracing #1. Use this to show spontaneous irregular heart.
4:23 P. M. Tracing #2.
4:26 P. M. Tracing #3.
4:30 P. M. to 4:35 Changed both rinsing three times.
4:36 P. M. Tracing #4.
4:39 P. M. Tracing #5.
4:47 P. M. to 4:52 Changed both rinsing three times.
4:52 P. M. Tracing #6.
4:55 P. M. Tracing #7.
5:12 P. M. Tracing #8 - auricle was beating at time but ventricle wasn't and here no beat was recorded on drum.
7:00 P. M. Put in 12 eggs marked "30".

#80. Put in July 26, 1926. (~~or 176~~) - 99 hours old)
7:35 P. M. Took out embryo in 20 cc L. L. Yolk broken.
7:45 P. M. Tracing #1 - 90 beats per minute.
7:48 P. M. Added 1 drop of .001% Atrppine.
7:52 P. M. First½tracing #2 - 75 beats per minute.
7:54 P. M. Added 1 drop of .001% Atropine.
7:55 P. M. Rest of tracing #2 - 165 beats per minute followed by 50 beats per minute.
7:58 P. M. Tracing #3.
7:59 P. M. to 8:04 Rapidly changed both 4 times
8:08 P. M. Tracing #4.
8:10 P. M. Tracing #5.
8:20 P. M. Tracing #6.

#81 or #175. Put in July 26, 1926. 101 hours old.
8:40 P. M. Took out embryo in 20 cc L. L.

8:49 P. M. Tracing - 60 beats per minute.
8:54 P. M. Added 2 drops of .001% Atropine.
8:58 P. M. Added 2 drops of .001% Atropine.
9:02 P. M. Added 4 drops of .001% Atropine.
9:06 P. M. Added 1 drop of .01% Atropine.
9:10 P. M. Added 2 drops of .01% Atropine.
9:14 P. M. 4 drops of .01% Atropine.
9:15 P. M. Tracing #2. Any apparent irregularities are due to looking up - 35 beats per minute - to see if marker was still on drum perfectly even.
9:19 P. M. Added 1 drop of .1% Atropine
9:22 P. M. Tracing #3
9:24 P. M. Tracing #4
9:26 to 9:31 P. M. Changed bath 4 times
9:32 P. M. Tracing #5
9:34 P. M. Tracing #6
9:46 P. M. Tracing #7

August 1, 1926
8:00 P. M. Put in 6 eggs marked "1".

August 2, 1926

#82. Put in July 26, 1926
9:37 A. M. Took out in 2 cc of E. L.
9:45 A. M. Before this heart could be observed beat and blood to flow. At this time they ceased.

#83. Put in July 30, 1926. 63 hours old. ~~(or #176)~~
10:05 A. M. Taken out in 20 cc of L. L.
10:28 A. M. Tracing #1, 105 beats per minute.
10:31 A. M. Added 1 drop of .001% Atropine.
10:34 A. M. Tracing #2
10:37 A. M. Added 2 drops of .001% Atropine
10:42 A. M. Added 3 drops of .001% Atropine
10:47 A. M. Added 1 drop of .01% Atropine
10:50 A. M. Tracing #3
10:54 A. M. Added 1 drop of .01% Atropine
10:59 A. M. Added 1 drop of .01% Atropine
11:03 A. M. Added 2 drops of .01% Atropine
11:10 A. M. Added 3 drops of .01% Atropine
11:11 A. M. Tracing #4
11:14 A. M. Added 1 drop of .1% Atropine

11:18 A. M. Added 1 drop of .1% Atropine
11:23 A. M. " 1 " " " "
11:28 A. M. Tracing #5
11:34 A. M. Added 5 drops of .1% Atropine
11:38 A. M. Tracing #6
11:41 A. M. Added 10 drops of .1% Atropine
11:50 A. M. Tracing #7 - 90 beats per minute
(Atropine had no effect on this embryo. Not even toxic)

#84 or #177. Put in July 30 69 hours old
3:37 P. M. Took out embryo in 20 cc of L. L. solution
3:52 P. M. Tracing #1 - 120 beats per minute
3:54 P. M. Added 1 drop of .001% Atropine
3:58 P. M. Added 1 drop of .01% Atropine
4:04 P. M. Tracing #2 - 105 beats per minute
4:09 P. M. Added 1 drop of .1% Atropine
4:12 P. M. Tracing #3 - 105 beats per minute
4:15 P. M. Add 5 drops of .1% Atropine
4:18 P. M. Tracing #4 - 110 beats per minute
4:21 P. M. Added 10 drops of .1% Atropine
4:24 P. M. Tracing #5 - 105 beats per minute
4:27 P. M. Added 10 drops of .1% Atropine
4:33 P. M. Tracing #6. 110 beats per minute

#85. Put in July 30, 1926 70 hour embryo Not fully developed. Hardly any blood
5:00 P. M. Took out embryo in 20 cc of L. L. Blood was circulating.
5:13 P. M. Added 10 drops of .1% Atropine
5:30 P. M. Heart just as regular as ever

#86. Put in July 30, 1926.
6:55 P. M. Infertile

#87. Put in July 30, 1926.
7:00 P. M. Monster embryo with few blood islands

August 3, 1926

10:00 A. M. Put 2 dozen eggs in incubator

#88. Put in July 30, 1926
10:45 A. M. Monster embryo with few blood islands. Probably this was living day before and was similar to those taken out then. Something evidently happened to the blood forming mechanism.

#89. Put in July 30, 1926
10:50 A. M. Infertile

#90 (~~or 178~~) 88 hours old. Put in July 30, 1926.
10:55 A. M. Taken out in 20 cc of L. L. solution
11:07 A. M. ½ of tracing #1 - 95 beats per minute
11:20 A. M. Last ½ of tracing #1 - 95 beats per minute
11:26 A. M. Added 1 drop of .1% Atropine
11:28 A. M. Tracing #2 - 115 beats per minute
11:30 A. M. ½ of tracing #3
11:33 A. M. Added 10 drops of .1% Atropine
11:34½ A. M. Last of tracing #3
11:36 A. M. Tracing #4
11:41
to A. M. Changed bath 4 times
11:46
11:47 A. M. More regular. Is effect due to new
11:49 A. M. solution or to removal of Atropine?
12:00 A. M. Tracing #7

#91 (~~or #175~~) 91 hour chick. Put in July 30, 1926
2:20 Embryo out in 5 cc of L. L. Yolk broke
2:32 P. M. Tracing #1 - 115 beats per minute
2:35 P. M. 10 drops of .1% Atropine
2:38 P. M. Tracing #2 - 110 beats per minute
2:40 P. M. Tracing #3
2:44 P. M. Added 20 drops of .1% Atropine
2:46 P. M. Tracing #4 - 120 beats per minute
2:55 P. M. Tracing #5 - 115 beats per minute

#92. Put in July 30, 1926
7:55 P. M. Blood islands only

#93. Put in July 30, 1926
8:00 P. M. Infertile

#94. Put in July 30, 1926
8:05 P. M. Blood islands only

#95. Put in July 26, 1926
8:10 P. M. Taken out - Heart cannot be observed to beat but blood can be seen circulating
8:35 P. M. Heart had spells of not beating

Put 4 eggs in incubator marked "3" - 9:00 P. M.

#96. Put in July 26, 1926
9:05 P. M. Yolk broken in taking out. Kept as a normal

August 4, 1926

#97. Put in July 26, 1926
10:24 A. M. Yolk broken. Took out in 10 cc of L. L.
10:30 A. M. Circulation present
10:34 A. M. Added 10 drops of .1% Strychnine
10:55 A. M. Movements were observed during the time before this which might have been made by the embryo spontaneously. They were not marked. They may have been cuased by air current.

#98. Put in July 26, 1926
11:15 P. M. Took out in 20 cc of L. L. solution, Yolk not broken.
11:20-11:25 P. M. Blood can not be observed to be circulating.
11:27 P. M. Added 50 drops of .1% Strychnine
Until 11:42 P. M.-No body movements could be observed. With no circulation the strychnine might have a hard time getting to the CNS or the CNS might be dead.

#99. Put in August 1, 1926
1:55 P. M. Infertile

#100 ~~(or #130)~~ 66 hour embryo. Put in August 1, 1926.
1:40 P. M. Taken out in 20 cc of L. L.
1:46 P. M. Tracing #1 - 125 beats per minute
1:49 P. M. Added 20 drops of .1% Strychnine
1:52 P. M. Tracing #2 - 70 beats per minute
1:54½ P. M. Tracing #3 - 60 beats per minute
2:01 P. M. Added 30 drops of .1% strychnine
2:03 P. M. Tracing #4
2:05½ P. M. first ½ tracing #5 - 55 beats per minute
2:12 P. M. Added 50 drops of .1% Strychnine
2:21 P. M. Added 100 drops of .1% Strychnine
2:22 P. M. Last of tracing #5
2:28
to P. M. Changed both 4 times
2:33
2:34 P. M. Tracing #6 - 110 beats per minute
2:41 P. M. Tracing #7 - 55 beats per minute

The above experiment would lead one to believe there is no C.N.S. connection to the heart. Strychnine should knock out the Central Nervous System centers

immediately and the heart should stop immediately. The reduction in heart rate 3 minutes after first addition of strychnine may be only incidental or may be due to direct action on heart muscle or conducting mechanism inside heart. If so, there ought to be an increased effect on addition of more strychnine; which there wasn't.

#101 or #181. Put in August 1, 1926
3:15 P. M. Taken out in 20 cc L. L.
3:20 P. M. Tracing - 80 beats to a minute
3:23 P. M. Added 1 drop of .1% Strychnine
3:34 P. M. Added 100 drops of .1% Strychnine
3:35 P. M. Tracing #2 - 70 beats per minute
3:37½ P. M. Tracing #3 - 50 beats per minute
3:48 P. M. Tracing #4 - 105 beats per minute

August 5, 1926

#102 Put in August 1, 1926
8:52 A. M. ruined in taking out

#103 or #182 83 hour chick embryo. Put in August 1, 1926.
9:00 A. M. Taken out in 20 cc L. L. solution
9:11 A. M. Tracing #1 - 90 beats per minute
9:16 A. M. Added 50 drops of .1% strychnine
9:19 A. M. Tracing #2 - 55 beats per minute
9:22 to 9:27 A. M. Changed bath 4 times
9:29 A. M. Tracing #4 - slightly irregular
9:42 A. M. Added 5 cc of .1% strychnine - heated to about 35°C.
9:43 A. M. Tracing #5
9:45½ A. M. Tracing #6
9:57½ A. M. Tracing #7 - observed during the 5 minutes previous to be as regular as shown in this tracing.
10:03 A. M. Decapitated embryo - must have cut into heart too because it stopped beating and hemorrhage was observed around it.

#104 Put in August, 1926.
11:20 A. M. Blood islands only.

#105. Put in August 3, 1926
1:20 P. M. Took out in 20 cc L. L. Decapitated. Heart beat for a while but when got it under the microscope it was either not beating or else all blood had run out so it could not be observed to beat.

#106 Put in August 3, 1926
1:45 P. M. Infertile

#107 Put in August 3, 1926
2:30 P. M. Infertile

#108 or #183 Put in August 3, 1926. 53 hours old.
2:45 P. M. Took out embryo in 20 cc L. L. solution.
3:15 P. M. Tracing #1
3:18½ P. M. Added 1 drop of .1% coffeine
3:23 P. M. Tracing #2
3:27 P. M. Added 10 drops of .1% coffeine
3:31 P. M. Tracing #3
3:37 P. M. Added 100 drops of .1% coffeine
3:38 P. M. Tracing #4
3:56 P. M. Tracing #5

August 7, 1926

#109 Put in August 3, 1926
8:35 A. M. Took out embryo in 20 cc of L. L.
8:57 A. M. Decapitated
9:01 A. M. Tracing #1 - regular all way through but very hard to see with blood out.
9:04 A. M. Heart was not beating.
9:08 A. M. Gave physical irritation to heart with glass rod. It began to beat but blood did not circulate.
9:12 A. M. Tracing #2 - Both auricle and ventricle happened to have extra beats occassioned but I am not sure.
9:25 A. M. Had not beaten for 2 or 3 minutes.

Way heart acted resembled very much that of earlier embryoes which were separated from the yolk and blood was allowed to escape from blood vessels.

To work efficiently I believe heart needs blood to pump.

#110 Put in August 3, 1926
10:50 A. M. Ruined in decapitating it.

#111 Put in August 3, 1926
11:10 A. M. Ruined in decapitating it.

#112 Put in August 3, 1926
11:25 A. M. Ruined in decapitating.

#113. Put in August 3, 1926
11:55 took out embryo and ligated head.
12:28 A. M. tracing #1 - soon stopped beating.

#114. Put in August 3, 1926
2:45 P. M. Infertile

#115. Put in August 3, 1926
2:55 P. M. Infertile

#116. Put in August 3, 1926
3:00 perfect embryo with beating heart but no blood present except a few islands at periphery. No blood circulating. Might have some bearing on theory of blood formation.

#117. Put in August 3, 1926
3:20 P. M. Stuck to shell and yolk broke. Decapitated anyhow for technic.

#118. Put in August 3, 1926
3:30 P. M. Broke yolk in taking out. Decapitated. Hemorrhage. Heart stopped.

#119. Put in August 3, 1926
4:00 P. M. Taken out. Decapitated but heart stopped.

8:00 P. M. Put in 1 dozen eggs marked "7".

August 9, 1926

9:00 A. M. Put in 2 dozen eggs marked "9".

#120. Put in August 3, 1926 - 9:00 P. M.

2:07 P. M. Took out embryo with all membranes but minus yolk in 20 cc L. L. solution.
2:32 P. M. Heart was not beating all the time so no observation was taken.

#121. Put in August 7, 1926
2:50 P. M. Monster embryo

#122. Put in August 7, 1926. 43 hour embryoes
2:57 P. M. taken out in 20 cc of L. L. solution
3:09 P. M. Tracing #1
3:12 P. M. Added 10 drops of F. digitalis
3:18 P. M. Tracing #2
3:23 P. M. Added 100 drops of Fr. digitalis
3:43 P. M. Removed solution. Heart had stopped beating and had left tincture on twenty minutes. It is not possible to see the heart when so much tincture is present in solution. Evidently either digitalis or the alcohol present in the tincture stopped the heart.

#123. 44 hours old.
4:20 P. M. Took out embryo in 20 cc of L.L. solution.
4:39 P. M. Tracing #1
4:42 P. M. Added 110 drops of 95 % undenatured alcohol
4:45 P. M. Heart was not beating

#124 (~~or #104. 71 hours~~ should be 47 hours) Put in August 7, 1926
7:12 Took out into 20 cc of L. L. solution
7:40 Tracing #1
7:47 Added to it ten drops of either (to bath)
7:48 Tracing #2
7:53 to 7:58 Changed both 4 times
7:58 Tracing #3
8:07 Tracing #4

#125
8:25 Infertile

#126
8:33 Infertile

August 10, 1926

7:50 AM. Found over 3 dozen eggs soft boiled. Batteries went dead during the night.

9:00 A. M. Put in 1 dozen eggs

August 13, 1926

#127 72 hours Put in August 10, 1926
9:04 A. M. Taken out in 20 cc of L. L. solution
9:26 A. M. Yolk broken; was discarded as unsuitable because could not see heart on account of yolk floating around.

#128 or #185 73 hours Put in August 10, 1926
9:40 A. M. Taken out in 20 cc L. L. solution
9:58 A. M. Tracing #1
10:10 A. M. Added 5 drops of chloroform
10:14 A. M. Tracing #2
10:18 A. M. Added 20 drops of chloroform
10:19 A. M. Tracing #3 - heart perfectly regular but slow
10:29 A. M. Tracing #4

#129. (T.)
Put in incubator December 6, at 1:30 P. M.
Taken out incubator December 8, at 3:20 P. M.
50 hours old - at room temperature
On yolk in 20 cc L. L. solution @ 22°C

A. 4:57 P. M. Start line #1
4:59 P. M. Start line #2
4:59½ P. M. Added dose diptheria toxin
5:01 P. M. Start line #3
5:03 P. M. Start line #4
5:03½ P. M. Heart getting paler
5:05½ P. M. Start line #5
5:08 P. M. Start line #6

B. 5:11 P. M. Start line #1
5:12½ P. M. Stop in middle line #1.
5:17 P. M. Start middle line #1
5:19 P. M. (about) stop line #1
8:10 P. M. Start line #3
8:10½ P. M. First effect line #3
8:12 P. M. Start line #4
8:14½ P. M. Stop line #5
10:00 P. M. Still living

December 9, 8 A. M., still beating. No pronounced; at least, lethal effect.

#130 (T2)
Put in December 6, 1926 at 1:30 P. M.
Taken out December 8, at 3:30 P. M.
50 hours - With yolk in 20 cc Locke-Lewis solution at room temperature 22°C
6:05 P. M. Start line #1
6:07 1/8 P. M. Start line #2
6:07 3/4 P. M. Added L+ dose diptheria toxin
7:53 1/8 P. M. Start line #3
8:17 P. M. Observed to be arrhymethic
10:00 P. M. Still living

December 9, 1926 - at 8:00 A. M. - still beating

#131 (T3) Control
Put in December 6, 1926 at 1:30 P. M.
Taken out incubator December 8, at 3:30 P. M.
50 hours - With yolk in 20 cc of Locke-Lewis solution at room temperature @ 22. Washed three times to remove white.
10:00 P. M. Still living
Nov. 9, 1926 - at 8:00 A. M. - dead

#132 (T4)
Put in December 7, 1926 at 8:30 A. M.
Taken out of incubator December 10, at 3:30 P. M.
79 hours old. With yolk in 20 cc Locke-Lewis solution at room temperature. Ammonic sac torn, at 23°C.
7:30 P. M. Added L+ dose diptheria toxin
9:40 P. M. No change noted up to this time
December 11, 1926 - 9:30 A. M. - one beat occasionally

#133 (T5)
Put in December 7, 1926 at 8:30 A. M.
Taken out incubator December 10, 1926 at 3:30 P. M.
79 hours. With yolk in 20 cc Locke-Lewis solution at room temperature @23°C.
7:30 P. M. Added L+ dose diptheria toxin
9:40 P. M. No change noted up to this time
December 11, 1926, at 9:30 A. M. - beating strongly and regularly

#134 (T6) and #135 (T7) Control
Put in incubator December 7, 1926 at 8:30 A. M.
Taken out incubator December 10, at 3:30 P. M.
79 hours. With yolk in 20 cc Locke-Lewis solution at room temperature @23°C.
December 11, 1926 - at 9:30 A. M. - beating strongly and vigorously with regularity

#136 (T8)
Put in incubator December 8, 1926 at 8:30 A. M. Taken out incubator December 11, at 4:30 P. M. 80 hours. Detached from yolk in watch glass with 5 cc Locke-Lewis solution at room temperature @21C.
7:06 P. M. Added L+ dose diptheria toxin
9:20 P. M. Mending of a pause of over 2 minutes; the first irregularity observed
11:00 P. M. Still beating at time just like control

#137 (T9)
Put in incubator December 8, 1926 at 8:30 A. M. Taken out incubator December 11, at 4:30 P. M. 80 hours. Detached from yolk in watch glass with 5 cc Locke-Lewis solution at room temperature @21°C
7:06 P. M. Added L+ dose diptheria toxin
7:11½ P. M. Heart stopped; first irregularity noted
7:13½ P. M. Started again - seemed more rapid - should have taken a normal so could compare
7:26 P. M. Stopped
7:27 P. M. Started again
7:30 to 7:32 P. M. A stopped period
11:00 P. M. Still beating at times like control

#138 (T10) Control
Put in incubator December 8, 1926 at 8:30 A. M. Taken out incubator December 11, at 4:30 P. M. 80 hours. Detached from yolk in watch glass with 5 cc Locke-Lewis solution at room temperature. @21°C.
7:06 P. M. Perfectly regular
11:00 P. M. Beating occassionally

#139 (T11) to #148 (T20)
Put in incubator December 10, 1926 at 8:30 A. M. Taken out of incubator December 16, at 8:00 P. M. 153½ hours old. Embryoes cut off from membranes and some with just heart cut out. In 10 cc Locke-Lewis solution in watch glass. @22°C. (T11)
9:00 P. M. Only one embryo with heart beating. Stroked medulla region with probe and heart stopped. After a while it started again. Stroked medulla region and heart stopped again. Maybe coincidence. Might be medulla was injured

by others when removing them to watch glass. On the face of things however, it looks like isolate embryoes at room temperature at this age will not live. The heart did not start up again.

#149 (T21)
Put in incubator December 14, 1926, at 8:30 A. M. Taken out of incubator December 17, at 8:00 P. M. 82 hours. Cut off from yolk in 5 cc Locke-Lewis solution at room temperature @22.
8:22 P. M. Added L+ dose diptheria toxin in .18 cc fluid.
8:40 P. M. Observed to have stopped and did not beat again till 10:03.

#150 (T22)
Put in incubator December 14, 1926, at 8:30 A. M. Taken out of incubator December 17, at 8:00 A. M. 82 hours. Cut off from yolk. In 5 cc Locke-Lewis solution at room temperature. @22°C.
8:22 P. M. added L+ dose diptheria toxin in 0.18 cc of fluid.
8:38 P. M. 78 beats per minute regular
9:03 P. M. 78 beats per minute regular
10:03 P. M. 84 beats per minute regular

#151 (T23)
Put in incubator December 14, 1926 at 8:30 A. M. Taken out of incubator, December 17, at 8:00 P. M. 82 hours. Cut off from yolk. In 5 cc Locke-Lewis solution at room temperature. @22
8:22 P. M. Added 0.18 cc diptheria toxin containing L+ dose.
8:35 P. M. 46 beats to a minute regular
8:41 P. M. Observed to have stopped
8:43 P. M. Started beating again
8:46 P. M. Heart observed to be stopped
8:47½ P. M. Heart beating - beat at irregular intervals until -
9:00 P. M. Stopped beating
9:06 P. M. Started beating
9:06½ P. M. Stopped beating
9:14 P. M. Nineteen beats then stopped
9:15½ P. M. A few beats then stopped
9:18 P. M. Bubbled O2 through solution
9:20 P. M. Heart began

Bubbling O_2 rather rapidly through solution made the heart come back to normal and beat regularly for ten minutes. This embryo had some blood vessels torn so that evidently circulation was not adequate to bring O_2 to the heart while in T22 vessel was intact and no O_2 was needed to be bubbled in.

10:03 P. M. Beats at times without O_2.

#152 (T24)
Put in incubator December 14, 1926 at 8:30 A. M.
Taken out of incubator December 17, at 8:00 P. M.
82 hours old. Taken off from yolk in 5 cc Locke-Lewis solution at room temperature. @22
8:22 P. M. Added 0.18 cc diptheria toxin containing L+ dose.
8:37 P. M. Pause first noted and is apparently dead; did not beat again up to 10:03.

#153
Put in incubator January 6, 1926 at 8:00 A. M.
Taken out of incubator January 8, at 1:00 P. M.
53 hours old in regular 37.5°C incubator. On yolk in 20 cc Locke-Lewis solution at room temperature. @20°C.
3:45 P. M. Tracing #1 - 10 beats to the minute
3:56½ P. M. Added 1 drop of .1% Atropine Sulfate
3:58 P. M. Line 2 - first part - 10 beats to the minute
4:13-4:19 P. M. Line 2 - second part - 10 beats to the minute. Regular throughout - any irregularity in tracing mechanical-time marker stopped working at times but speed was uniform.
4:40-4:47½ P. M. First of line #3 - 10 beats per minute
4:49 P. M. 10 drops of .1% Atropine Sulfate then started drum - 10 beats per minute.
4:52 P. M. Stopped drum after mechanical difficulties
4:55 to 5:03½ P. M. Last of tracing #3 - 5:02
5:03 - 10 beats per minute
7:13 to 7:20 P. M. First part tracing #4. Regular. Time marker not indicating all the time but drum was running at a uniform speed.
5 beats to a minute.

January 9, 1926, at 12:30 (noon), heart had stopped beating. This embryo was not a strong one to begin with. No artificial light was used and the beat remained constant (10 per minute) through out the experiment in the afternoon when the drug was added.

#154-#165
Put in incubation, January 6, 1926 at 8:00 A. M. Taken out of incubator January 8, at 1:00 P. M. 53 hours old. Not developed so beating heart was present. Eggs were too old and not saved for this purpose.

#166
Put in incubator January 6, 1926, at 8:00 A. M. Taken out of incubator January 8, at 8:00 P. M. 60 hours old in regular 37.5° incubation. On yolk in 20 cc Locke-Lewis at room temperature. (22°C)
8:31 to 8:35 P. M. Normal first part of line 1. 12 beats per minute.
8:36 P. M. Added 1 drop of .1% Atropine Sulfate
8:36 to 8:44 P. M. Rest of line 1 (15 beats per minute between 8:43 to 8:44)
8:45 P. M. Added 10 drops of .1% Atropine Sulfate
8:45 P. M. to about 8:58 P. M. Line 2. Temperature 24°C (20 beats per minute - 8:56½ - 8:51)
January 9, 1926 at 12:30 (noon) heart had stopped beating. This embryo was not a strong one to begin with.

#167
Put in incubator January 6, 1927, at 8:00 A. M. Taken out of incubator January 8, at 8:00 P. M. 60 hours old in regular 37.5°C)
9:09 - 9:12 P. M. Named first part line #1
13 beats per minute
9:13 P. M. Added 1 drop of .1% Atropine Sulfate
9:13-9:21 rest of line #1: 9:13-9:14 - 14 beats per minute; 9:14-9:15 - 16 beats per minute; 9:15-9:16 - 15 beats per minute; 9:16-9:17 - 15 beats per minute.
9:24 P. M. Added 10 drops of .1% Atropine Sulfate
Count per minute beginning 9:24: 20, 19, 19, 19, 20,22,20,21,22,22,21,22,23,23 per minute
9:24 to 9:35 P. M. Line 2. Rate looks slightly

to be increased but temperature rose from 22°C to 24°C due to electric light which probably accounts for any acceleration. January 9, 1927, at 12:30 (noon) heart was stopped. These embryoes are not as strongly developed as others which would beat 48 hours.

#168, - #173. Incompletely developed embryoes Put in incubator (39°C) February 25, 1927 at 8:30 A. M. Taken out of incubator February 27, 1927 at 11:30 A. M. 51 hours old. On yolk in 20 cc Locke-Lewis solution

#174 (control)
Put in incubator (39°C) February 25, 1927 at 8:30 A. M. Taken out of incubator February 27, 1927 at 11:30 A. M. 51 hours old. On yolk in 20 cc Locke-Lewis at 23°C. Room temperature
1:10 P. M. To first part of tracing 1 regular, 23°C, 1:15 P. M.
4:16 to 4:20 P. M. Second part of tracing #1. @24.25°C.
February 28, 1927 could be seen to be beating but bloodless heart could not be seen well enough to count beats accurately at 11:00 A. M.

#175 (Control)
Put in incubator (39°C) February 25, 1927 at 8:30 A. M. Taken out of incubator Feb. 27, 1927 at 11:30 A. M. 51 hours old on yolk in 20 cc Locke-Lewis solution at room temperature.
1:27 P. M. to 1:33 P. M. First part of tracing 1 regular 23°C.
4:23 to 4:28 P. M. Second part of tracing #1 @24.25°C. February 28, 1927 at 11:05 A. M. Heart could not be seen.

#176
Put in incubator (39°C) February 25, 1927 at 8:30 A. M.) Taken out incubator February 27, 1927 at 11:30 A. M. 51 hours old. On yolk in 20 cc Locke-Lewis at room temperature 1:50 to 1:54 P. M. First part of tracing #1 1:57½ P. M. Added 1 drop of 1% Atropine Sulfate

1:59 to 2:05 P. M. Second part of tracing #1 @23°C.
3:25 to 3:32 P. M. First part of tracing #2 @24°C (it is on graph for #177)
3:34 to 339 P. M. Rest of tracing #2 on graph for #177
3:34 P. M. Added 10 drops of 1% Atropine Sulfate @24.25°C
February 28, 1927 -
11:12 to 11:27 A. M. Tracing #2 - regular. Any irregularity shown on drum is due to looking up to see if marker is on drum. @22°C. This embryo for twenty hours has been in contact with eleven times the therapentic dose of Atropine Sulfate for an adult human.

#177
Put in incubator (39°C) February 25, 1927 at 8:30 A. M. Taken out of incubator, February 27, 1927 at 11:30 A. M. 51 hours old. On yolk in 20 cc Locke-Lewis solution at room temperature
2:58 to 3:01 P. M. First part of tracing #1 @23.5°C
3:03 P. M. Added 1 drop of 1% Atropine Sulfate
3:04½ to 3:09½ P. M. Second part of tracing #1 @ 23.5°C
3:52 to 3:57½ P. M. First part of tracing #3. @24.5°C
4:00 P. M. Added 10 drops of 1% Atropine Sulfate
4:02 to 4:07 P. M. Second part tracing #3. @ 24.5°C
February 28, 1927 at 11:35 A. M. Heart could not be observed so it is not known whether or not it was beating

#178-181. Embryoes that to be alright later proved to be unsatisfactory before starting to use them
Put in incubator 39°C, February 25, 1927 at 8:30 A. M. Taken out of incubator, February 27, 1927 at 11:30 A. M. 51 hours old. On yolk in 20 cc Locke-Lewis at room temperature

#182 to #185 Yolk torn so badly they could not be used
Put in incubator (39°C) February 26, 1927 at 9:00 A. M. Taken out of incubator March 1, 1927 at 8:00 A. M. 71 hours old.

#186 (control) Yolk slightly torn
Put in incubator (39°C) February 26, 1927 at 9 A. M. Taken out of incubator March 1, 1927 at 8 A. M. 71 hours old. On yolk in 20 cc of Locke-Lewis solution at room temperature 10:33 to 10:37 A. M. First part of tracing, @22°C
March 2, 1927 at 8:30 A. M. Heart could not be observed to be beating.

#187 (control)
Put in incubator (39°C) February 26, 1927 at 9 A. M. Taken out of incubator March 1, 1927 at 8 A. M. 71 hours old. On yolk in 20 cc Locke-Lewis solution at room temperature 11:17 to 11:22 A. M. First part of tracing #1 @21°C.
March 2, 1927 at 8:30 A. M. Heart could not be observed to be beating

#188
Put in incubator (39°C) February 26, 1927 at 9:00 A. M. Taken out of incubator March 1, 1927 at 8:00 A. M. 71 hours old. On yolk in 20 cc Locke-Lewis solution at room temperature
11:32 to 11:37 A. M. First part of tracing #1 @ 20.5°C
11:40 A. M. Added 50 mgs of Atropine Sulfate (5 cc of 1% solution)
11:40 to 11:50 A. M. Second part of tracing #1 A 20.5°C. Perfectly regular. Any apparent irregularity is mechanical
March 2, 1927, at 8:30 A. M., heart could not be observed to be beating

#189
Put in incubator (39°C) February 26, 1927 at 9 A. M. Taken out of incubator March 1, 1927 at 8 A. M. 71 hours old. On yolk in 20 cc Locke-Lewis solution at room temperature
3:57 to 4:02 P. M. First part of tracing #1 @22°C

March 2, 1927

9:11 to 9:16 A. M. Third part of tracing #1 @23°C

9:20 A. M. Added 0.2 cc of 1-10 rabbit serum
4:20 P. M. Changed bath 3 times and added 1 cc of 1$ Atropine. Yolk badly broken
4:34-4:41 P. M. First part of tracing #2. Irregular
4:58-5:07½ P. M. Regular now
5:13½ P. M. Added 1.2 cc of 1-10,000 anti-rabbit (1-10,000 titre)
5:14 to 5:25 P. M. First part of tracing #3
5:30 P. M. Changed bath 3 times and added 1 cc of 1% Atropine Sulfate
5:38(?) to 5:44 P. M. Rest of tracing #3 @26°
5:45 P. M. Added 1.2 cc of 1-10 anti rabbit
5:45 to 5:50 P. M. Tracing #4

#190 - #197
Put in March 5, 1927 at 9 A. M.
Taken out incubator (39°C) March 7, at 9 P. M.
60 hours old
March 8, 1927 at 8:30 A. M. Four (4) (190-193) embryoes were still beating and in good condition but were ruined learning a new technical procedure

#198-#203
Put in incubator (39°C) March 7, 1927 at 8:30 A. M. Taken out of incubator (34°C) March 8, 1921 at 8:00 P. M. 36 hours but incubator had cooled down. No embryoes could be found

#204-209
Put in incubator March 7, 1927 at 8:30 A. M.
Taken out of incubator March 9, 1927 at 7 P. M.
Only two embryoes were well enough developed to even make out. The incubator cooling down had inhibited their growth so even if they had developed sufficiently for observation they could not be used because then age could not be known

#210-#215
Put in incubator (37°C) March 8, 1927 at 8:00 P. M.
Taken out of incubator March 11, at 11:00 A. M.
63 hours old
Only two, had beating hearts. These hearts became irregular before they could be tested with drugs. Learned technic of parafin cups on them

#216
Put in incubator (38°C average) March 12 at 9:00 A. M. Taken out incubator March 15, at 9:00 A. M. 72 hours old. In cup in 20 cc Locke-Lewis solution at room temperature on yolk

March 16, 1927

8:41 to 8:46 A. M. First part of tracing #1. @21.75°C
8:50 A. M. Added 1 cc of 1% Atropine Sulfate to bath
8:50 to 9:01 A. M. Rest of tracing #1
9:01 to 9:04½ A. M. First part of tracing #2 @22.5°C
10:01 to 10:07 A. M. Second part of tracing #2 @24.5°C start and stop
3:45 to 3:59 P. M. Tracing #3 @22.75°C

#217

Put in incubator (av.38°C) March 12, 1927 at 9:00 A. M. Taken out of incubator, March 15, 1927 at 9:00 A. M. 72 hours old. In cup in 20 cc Locke-Lewis solution - room temperature

March 16, 1927

9:18 to 9:23 A. M. First part of tracing #1 @23°C
9:27 A. M. Added 5 cc of 1% Atropine Sulfate
9:27½ to 9:38½ A. M. Rest of tracing #1 @23.5°C
9:39 to 9:55 A. M. Tracing #2 (temperature rose 23.5°C to 24°C during tracing)
4:08 to 4:14 P. M. Tracing 3 irregular hearts. Hard to see; might have been beating regularly but could not be seen. @22°C

#218

Put in incubator (38°C) March 12 at 9:00 A. M. Taken out of incubator March 15 at 9 A. M. 72 hours old. In cup on yolk with 20 cc Locke-Lewis at room temperature
10:28 to 10:33 A. M. First part of tracing #1 @ 25.5°C
10:49 A. M. Added 3 cc of 1% Atropine Sulfate @25.5°C
10:49 to 11:00 A. M. Rest of tracing #1 at 25.5°C
11:00 to 11:15 A. M. Tracing #1 at 25.5°C (ended at @25°C)
4:20 to 4:34 P. M. Tracing 3 regular A 23°C

#219

Put in incubator (av. 38°C) March 12, 1927 at 9:00 A. M. Taken out of incubator March 15, 1927 at 9:00 A. M. 72 hours old. In cup on yolk with 20 cc Locke-Lewis solution at room temperature
February 16, 1927
11:34 to 11:38 A. M. First part of tracing #1 (Mechanical) Heart perfectly regular @25°C
11:39 A. M. Added 2 cc of 1% Atropine Sulfate
11:39 A. M. to 11:48 A. M. Rest of tracing #1 @ 25°C
11:48 A. M. to 12:03 P. M. Tracing #2 (began 25°C; end 24.5°C)
4:40 P. M. to 4:49 P. M. Tracing #3 @22.5°C

#220

Put in incubator March 15, 1927 at 8:30 A. M. Taken from out incubator (37.5°C) March 18, 1927 at 8:30 P. M. 84 hours old. In cup on yolk with 20 cc Locke-Lewis at room temperature

March 19, 1927

10:49 to 10:54 A. M. First part of tracing #1 @24°C
10:57 A. M. Added 5 cc of 1% Atropine Sulfate
10:58 A. M. to 11:09 A. M. rest of tracing #1. Began @ 24°C. Ended @23.5°C.
11:10 to 11:26 A. M. Tracing #2 @23.5°C.
6:55 to 7:05 P. M. Tracing #3 @25°C.

#221

Put in incubator (37.5°C) March 15, 1927 at 8:30 A. M. Taken out of incubator March 18, 1927 at 8:30 P. M. 84 hours old. In cup on yolk in 20 cc Locke-Lewis solution at room temperature
11:52 to 11:57 A. M. Tracing #1 @23.5°C
12:00 Noon Added 5 cc of 1% Atropine Sulfate solution
12:00½ to 12:09 P. M. Rest of tracing #1 Start 23.5°C and end 24°C.
12:10 to 12:27 P. M. Tracing #2 @24°C all time. Note drum did not have uniform rate of speed
7:12 P. M. to 7:17 P. M. Tracing #3. Had to withdraw cup containing yolk from beaker to observe about 25°C to 26°C.

#222

Put in incubator (37.5°C) March 15, 1927 at 8:30 A. M. Taken out of incubator March 18, 1927 at 8:30 P. M. Tested March 19, 1927. 84 hours old. In cup on yolk with 2 cc of Locke-Lewis solution at room temperature
March 19, 1927
12:58 to 1:02 P. M. Tracing #1 @24.25 began to 24.50°C ended.
1:05 to 1:15 P. M. Rest of tracing #1 @24.5°C all time
1:05 P. M. Added 5 cc of 1% Atropine Sulfate
1:15 to 1:31 P. M. Tracing #2 began at 24.5°C. Ended at 24.75°C. Very irregular
7:28 P. M. to 7:38 P. M. Tracing #3 @26°C. Slightly irregular. Would point to toxic drug effect in tracing 2 since previous embryoes which became spontaneously irregular never came back. Here is one which became very irregular after giving drug and came back in six hours to be almost completely regular.

HAVE QUIT USING WHALEY EGGS AND AM USING CLAWSON NOW.

#223 to #226. Infertile

#227 to #228 Ruined in taking out. 87 hours old

#229

Put in incubator (37.5°C) April 4, 1927 at 6:30 P. M. Taken out of incubator April 8, 1927 at 9:00 A. M. 87 hours old. In 20 cc Locke-Lewis solution at room temperature to begin on yolk
11:32 A. M. Start @240 C
8:19 P. M. Last of tracing #1 @25.5
8:59 P. M. to 9:12 P. M. Tracing #2 irregular at times @27°C
9:45 P. M. to 9:51 P. M. Tracing #3 @37°C irregular

#230

Put in incubator (37.5°C), April 4, 1927 at 6:30 P. M. Taken out of incubator, April 8, 1927 at 9:00 A. M. 87 hours old. In 2 cc

L Locke-Lewis solution in cup on yolk at room temperature to begin
11:44 A. M. Start and 11:54 stop - tracing one regular @ 24°C
11:57 A. M. Added 5 cc 1% Atropine Sulfate
11:57 A. M. to 12:04½ P. M. Rest of tracing #1
12:05 to 12:16 P. M. First part of tracing #2. At first mechanical irregular tracing but later had time pauses. @25°C.
6:58 to 7:01 P. M. Second part of tracing two heart stopped at times. @ 25°C.
7:16 to 7:21 P. M. Rest of tracing two perfectly regulated . @26.5°C.
7:21 to 7:30 P. M. First part tracing #3 perfectly regular, any irregularity due to looking up.
7:56 P. M. Start last part of tracing 3 ~~(perf)~~ C 37°C (constant to ½ degree)

#231
Put in incubator (37.5°C) April 4, 1927 at 6:30 P. M. Taken out of incubator April 8, 1927 at 9:00 A. M. 87 hour old. On yolk in 20 cc Locke-Lewis solution.
10:33 P. M. to 10:38 P. M. First part of tracing #1 @25.5°C.
10:47 P. M. Added 5 cc of 1% Pilocarpine S04
10:47 to 10:58 P. M. Rest of tracing of #1. First effect at 10:50 P. M. @25.5°C.
11:03 to 11:06 P. M. First part of line #2 @ 25.5°C.
11:19 to 11:25 P. M. Second part tracing #2. @37°C.

#232
Put in incubator (37.5°C) April 4, 1927 at 6:30 P. M. Taken out of incubator April 8, 1927 at 9:00 A. M. 87 hours old. In 20 cc Locke-Lewis
11:42 P. M. to 11:50 P. M. First part of tracing #1
11:50 P. M. Added 5 cc of 1% Pilocarpine S04
11:52 to 11:56 P. M. Rest of line #1
11:58½ to 12:01 P. M. First of line #2

#233
Put in incubator (39°C) April 5, 1927 at 9:00
Taken out of incubator (39°C)) April 8, 1927 at 7 P. M. 82 hours old. On yolk in 20 cc Locke-Lewis. Tested April 9, 1927
7:40 P. M. to 7:50 P. M. First part of tracing @27¼°C
7:53 P. M. Added 5 cc of 1% Pilocarpine S04

7:53 to 8:08 P. M. Tracing #2. First effect at 7:55 P. M. @ 27°C
8:36 P. M. (about) to 8:45 P. M. Tracing 3 @37°C

#234-#237. Good embryoes ruined for technic. Put in April 4, 1927

#238-#243. Infertile or maldeveloped. Put in April 5, 1927

#244-#248. Good embryo ruined for technic. Put in April 5, 1927

#249-#250. Infertile or maldeveloped. Put in April 6, 1927

#251-#259. Good embryoes ruined for technic. Put in April 6, 1927

#260

Put in incubator (39°), April 6, 1927 at 8:30 A. M. Taken out incubator April 9, 1927 at 7:30 P. M. 83 hours old. On yolk in 20 cc Locke-Lewis solution.
April 10, 1927
12:55 P. M. to 1:01 P. M. Tracing #1
Slightly irregular so was not used.

#261

Put in incubator (37.5°C) April 8, 1927 at 7:00 P. M. Taken out incubator April 12, 1927 at 8:00 A. M. 85 hours old. On yolk in 20 cc Locke-Lewis
10:32 A. M. to 10:41 A. M. First part of tracing #1 @24.5°C. Slightly irregular
10:57½ to 10:58½ A. M. Show part of tracing #2 @36°C
10:59½ A. M. to 11:00 A. M. Fast part of tracing 2 @ 36°C. Regular
11:03 added 5 cc of 1% Pilocarpine Sulfate
11:03 A. M. to 11:05 A. M. Line #3 @36°C
11:05 A. M. to 11:07 A. M. Line #4 @36°C
11:07 A. M. to 11:07½ A. M. First part of line @36°C
11:07½ A. M. to 11:15 A. M. Slow part of line #5. Heart regular again @36°C "anaphylactic effect" has passed off.
11:20 to 11:23 A. M. Changed both rinsing 3 times

Drum B

11:32 A. M. to 11:34 A. M. Tracing #1 . The normal after changing both @36°C

11:34 A. M. Added 5cc of 1% Pilocarpine Sulfate @36°C

11:34 to 11:35 A. M. Tracing #2 @36°C

11:36 A. M. to 11:38 A. M. Tracing #3 @36°C

11:38 A. M. to 11:40 A. M. Tracing #4. @ 36°C

11:38 A. M. to 11:40 A. M. Tracing #4. @ 36°C

11:40 A. M. to 11:42 A. M. Tracing #5 @ 36°C

Second time heart was "desensitized to toxic (or "anphylactic") effect of irregularly although heart was slowed.

Drum C

11:53 A. M. Added 5 cc of 1% $BaCl_2$

11:53 A. M. to 11:55 A. M. Tracing #1 @36°C

11:55 A. M. to 11:57 A. M. Tracing #2 @36°C

11:57 A. M. to 11:59 A. M. Tracing #3 @36°C

11:59 A. M. to 12:01 P. M. Tracing @36°C.

1:30 Stopped beating

#262

Put in incubator (37.5°C) April 8, 1927 at 7:00 P. M. Taken out of incubator April 12, 1927 at 8:00 A. M. 85 hours old. Cut off the yolk just before using. In 5 cc of bath.

7:36 P. M. to 7:41 P. M. normal @26°C. Heart slightly irregular

7:42 P. M. Added .0013 grams of ergotoxine with a resulting total bath of 5 cc. The ergotoxine came as hypodermic tablets and when dissolveed gave the appearance of gum acacia

7:42 P. M. to 7:52 P. M. Rest of line #1 Heart on the whole speedy. May or may not have been due to drugs.

#263-#272.

Infertile or maldeveloped. I here count those as maldeveloped which become irregular before I can work on them. Some times I can not work on them until 12 hours or more after removing from the incubator

The following eggs are H. C. Flory - white leghorn

#273-#283
Yielded 10 good embryoes. The yolk on two was broken in removing and the apparatus got~~ten~~ out of order before the others could be used.
Put in April 20, 1927 at 6:30 P. M.
Taken out April 23, 1927 at 9:30 A. M.

#284-#285
Two infertile eggs
Put in April 21, at 8:30 A. M.
Taken out April 23, at 8:30 P. M.

#286
Put incubator (37.5°C) April 21 at 8:30 A. M.
Taken out of incubator April 23, 1927 at 8:30 P. M. 60 hours old. On yolk in 20 cc Locke-Lewis solution in cup.
8:53 P. M. to 8:58 P. M. First of tracing @36°C
8:59 P. M. Added 5 cc of 1% Atropine S04
8:59 P. M. to 9:09 P. M. Rest of tracing @ 36°C.

#287
Put in incubator (37.5°C) April 21, 1927 at 8:30 A. M. Taken out of incubator April 23, 1927 at 8:30 P. M. 60 hours old. In yolk in 20 cc Locke-Lewis solution in cup
9:27 P. M. to 9:33 P. M. Tracing one - #1 @36°C
9:35 P. M. Added 5 cc of 1% Atropine Sulfate
9:35 P. M. to 9:45 P. M. @36°C.

#288-#289 Infertile
Put in April 21, 1927 at 8:30 A. M.
Taken out April 24, 1927 at 9:30 A. M.

#290
Put in incubator (37.5°C) April 21, 1927 at 8:30 A. M. Taken out of incubator April 24, 1927 at 9:30 A. M. 73 hours old. In 20 cc Locke-Lewis in cup.
10:50 to 10:55 A. M. First part of tracing #1. @36°C.
10:55 A. M. Added 5 cc of 1% Pilocarpine Sulfate @36°C.
10:55½ A. M. to 11:05½ A. M. Rest of tracing #1
6:30 P. M. Beats occasionally

#291
Put in incubator (37.5° C) April 21, 1927
8:30 A. M. Taken out of incubator
April 24, 1927 at 9:30 A. M. 73 hours old.
On yolk in cup in 20 cc Locke-Lewis solution.
11:37 A. M. to 11:42 A. M. Tracing #1.
First part @37°C.
11:42 A. M. Added 5 cc of 1% Pilocarpine
Sulfate @37°C
11:42½ to 11:52½ A. M. Rest of tracing #1.
Slowing but regular
6:30 P. M. Stopped.

#292 to #301
Good embryoes but could not be used since looked too young. Heart was just visable but should have been more developed at this age. Someone must have left the incubator door open overnight.
Put in April 22, 1927 at 8:30 A. M.
Taken out April 24, 1927, at 6:30 P. M.

#302
Put in incubator (37.5°C) April 23, 1927 at 9:00 A. M. Taken out of incubator April 26, 1927 at 8 A. M. 71 hours old. In cup in 20 cc Locke-Lewis solution.
9:45 A. M. to 9:50 A. M. Tracing one first part @36°C.
9:51½ A. M. Added 5cc of 1% Pilocarpine Sulfate @36°C.
9:52 to 10:02 A. M. Rest of tracing #1. @36°C.
11:45 A. M. to 11:50 A. M. Tracing #2. @ 36°C.

#303
Put in incubator (37.5° C) April 23, 1927 at 9 A. M. Taken out of incubator April 26, 1927 at 8 A. M. 71 hours old. In cup in 20 cc Locke-Lewis solution.
10:42 A. M. to 10:47 A. M. First part of tracing #1 normal @36°C.
10:47½ A. M. Added 5 cc of 1% of Pilocarpine Sulfate. @36°C.
10:48 A. M. to 10:57 A. M. Rest of tracing #1 @36° C.
12:01 P. M. to 12:06 P. M. Tracing #2 @36°C.

#304.
Put in incubator (37.5°C) April 23, 1927 at 9:00 A. M. Taken out of incubator April 26, 1927 at 8:00 A. M. 71 hours old. In cup in 20 cc Locke-Lewis solution.
11:14 A. M. to 11:19 A. M. Just part of tracing #1 @37°C.
11:19⅛ A. M. Added 5 cc of 1% Pilocarpine Sulfate @37°C.
11:20 A. M. to 11:33 A. M. Rest of tracing #1 @37°C.
12:15 P. M. to 12:20 P. M. Tracing #2 @37°C. Periodic fast and slow beating

#305-#307 Infertile - Put in April 23, 1927 at 9:00 A. M.

#308-#311 Were irregular or maldeveloped. Put in April 25, 1927 at 9:00 A. M.

#312
Put in incubator (37.5°C) April 25, 1927 at 8:30 A. M. Taken out of incubator 37.5°C, April 27, 1927 at 8:30 A. M. 48 hours old. On yolk in cup with 20 cc Locke-Lewis solution.
9:52 A. M. to 9:57 A. M. Tracing #1 - normal regular @36°C.
9:58 A. M. Added 5 cc of 1% Pilocarpine sulfate @36°C.
9:58 A. M. to 10:08 A. M. Tracing #2. No toxic effect. @36°C. At beginning a little fast due to stimulation of pouring in fluid. This appeared before drug could have penetrated.

#313 - #321
Embryoes maldeveloped. Put in incubator April 25, 1927 at 8:30 A. M.

#322 - #331
Put in April 26, 1927 at 8:00 A. M. Taken out April 28, 1927 at 8:00 A. M. Embryoes maldeveloped.

DISCUSSION

The demonstration by Sherwood (1) of cardiac anaphylaxis in chick embryoes ranging in incubator age around 48 to 72 hours without apparent nervous tissue in the heart raises the question of whether anaphylaxis affects the muscle cell directly or whether the phenomenon is due to some indirect effect.

Histological studies are quoted from Lillie (2): "The developement of cardiac nerves is of special interest on account of its bearing on the physiological problem of the origin of the heart beat. The heart of the chick begins to beat long before any nervous connection with the central nervous system can have been established; indeed, the rhythmical pulsation begins at about the stage of ten somites when the neural crest is yet undifferentiated, and no neuroblasts are to be found anywhere. Either, then, the heart beat is of muscular origin (myogenic), or, if of nervous origin, the nerve concerned must exist in the wall of the cardiac tube ad initio. The first trace of nerve cell is found in the heart of the chick about the sixth day. These cells are at the distal ends of branches of the vagus, with which they have grown

into the heart. Previous to this time these neuroblasts are found nearer to the vagus along the course of the arteries. There can be but little doubt that they have arisen from the vagus ganglion and that they reach the heart by migration. Such an origin has been demonstrated with great probability for all the known nervous elements of the heart of the chick. If any cardiac nervous elements arise in situ, they certainly remain undifferentiated until those which have a ganglionic origin have already entered the heart."

The results just given with drugs apparently indicate that in a certain percentage of embryoes a nervous-like mechanism appears in the chick heart on the third day. Variations in its appearance time may be due to the unreliability of incubator age as an index of the somatic age.

The smooth muscle reaction has recently come into prominence in the study of anaphylaxis. The question is raised as to whether these muscle cells act alone during anaphylaxis or is their action prompted by nervous stimuli. The motor innervation of smooth muscle and cardiac muscle is from the autonomic nervous system. Probably Sollman (10) has given us as clear and concise a review of this system as anyone. Pottenger (3) has studied this system from the standpoint of the

clinician and has made some very valuable observations. He has analyzed the symptoms of hay fever and asthma and has considered these anaphylactic in nature and explained these symptoms on the basis of parasympathetic stimulation. In addition to the evidence presented by Pottenger's observations, there is other which will support this theory. Atropine which paralyzes the parasympathetic innervation and epinephrin which stimulates the sympathetic, the physiological antagonist of the parasympathetic, has been used to prevent clinical anaphylaxis and in the treatment of asthma and hay fever. The latter two are forms of hypersensitiveness closely related to anaphylaxis. Stoland and Sherwood (4) have shown that Atropine Sulfate, which paralyzes the parasympathetic endings, prevents the characteristic anaphylactic response by contraction in uterine muscle strips of sensitized virgin guinea pigs. Auer (5) has shown that Atropine Sulphate would frequently prevent pulmonary manifestations of anaphylaxis in the sensitized guinea pig. The fact that only a part of the chick embryoes showed an alergic response (1) along with the fact that only a part were affected by Pilocarpine, Atropine, and Physostigmin suggest that a nervous mechanism is not entirely ruled out.

Both the sympathetic and parasympathetic innervate the viscera, including the heart. The parasympathetic acting through the vagus slows the heart, while its physiological antagonist, the sympathetic acting through branches from the inferior cervical ganglion and the stellate ganglion hasten the heart. If anaphylaxis is manifested as a stimulation of the parasympathetic then it would be logical to expect a slowing of the heart with long and overly full diastole, since this is what results characteristically from stimulation of t he parasympathetic. Such has been shown to be the result of anaphylaxis in chick embryoes (1) which were only three days old.

Gibbs (6) has worked with drugs selectively affecting the autonomic nervous system using the adult fowl. He found that Pilocarpine in the doses used had no demonstrable effect on the vagus of the fowl. Atropine was found to paralyze the vagus of the fowl but normally he found no vagus tone present. He concluded that Physostigmin in doses of 0.5 to 1 mgm. per kilo greatly prolongs the effect of vagus stimulation but does not itself initiate inhibition of the fowl's heart.

That the embryonic chick heart will beat without central nervous system connections was demonstrated by

Burrows and Suzuki (11). Using tissue cultures of the heart muscle from eleven and twelve day old chick embryoes, they found that Diphtheria Toxin in appropriate concentration would inhibit growth of such cultures. Diptheria Antitoxin would neutralize and remove such inhibiting effect. They state that the sensitiveness of the cells of chick embryoes to Diphtheria Toxin varies inversely with the age of the embryo. In this connection it is interesting to note that my embryonic chick hearts were younger than theirs and that I obtained no lethal effect with Diphtheria Toxin on them.

Any characteristic drug effect or toxic effect seemed to appear in embryoes around 71 hours or older. It is near this age that cardiac anaphylaxis appears. My results suggest that perhaps the same protoplasmic mechanism may be at least one factor in anaphylactic response. It is realized that toxic doses of these drugs may affect the heart muscle directly. This mechanism may consist of nervous tissue as it is now defined or may lead to the conception of a new kind of tissue found in embryoes which combines both nervous and muscular qualities. It is interesting to note that temperature affects anaphylaxis and sensitiveness

to drugs in the same way. Both occur in suitable embryoes at incubator temperature and both are markedly inhibited at the lower room temperature. Further work suggested by this is that the effects of drugs be first tried on the embryo and then passive anaphylaxis be tried on the same embryo. It would be of importance to establish if one can occur without the other occurring. This would tend to prove whether or not their mechanism is the same. In one instance, embryo #189, no apparent effect was obtained by Atropine Sulfate nor could passive anaphylactic response be produced. High titred immune sera was used with the standard technic. Room temperature was used during the experiment.

SUMMARY

Out of 331 eggs incubated, 174 were infertile or maldeveloped, 78 were used to gain a knowledge of technic, 9 were untreated and 70 were treated with pharmacological products. The experiments may be grouped into two classes, one at incubator temperature around 39 degrees C. and the other at room temperature around 22 degrees C. With the material and technic used a lethal effect was not demonstrated for Diptheria Toxin on the embryonic chick heart. Drugs seemed to have a marked effect at incubator temperature in embryoes around 71 hours and older. At room temperature drug effect was obtained only with massive doses in 71 hour or older chick embryoes. This was an apparent toxic effect. A much greater relative dosage of the drug was required at room temperature to get even a mild effect.

CONCLUSION

1. Some mechanism, either nervous or having some of the attributes of a nervous mechanism appears in chick embryoes around 71 hours of age. This is before any present histological method shows a nervous mechanism.

2. The appearance time for a mechanism susceptable to drugs is about the same as that for a mechanism giving cardiac anaphylaxis, altho it should be observed that some of the anaphylactic responses were obtained at a little earlier incubation age. This suggests that the anaphylactic response may come from the tissue itself or from early functioning nervous tissue in the sinus region.

3. The inhibiting effect of decrease in temperature is the same on either mechanism.

4. The effects of drug action are largely in accord with the findings of Gibbs (6) with certain interesting exceptions in the case of Pilocarpine and to some extent with Physostigmin.

5. It is believed that irregularities due to temperature change as suggested by the work of Lewis (8) were ruled out.

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