"On the 'Blaze-currents' of the Incubated Hen's Egg." By AUGUSTUS D. WALLER, M.D., F.R.S. Received July 17,—Read December 4, 1902.

(From the Physiological Laboratory of the University of London, S.W.)

In previous communications to the Society, on the Eyeball,* on the Skin,† and on Leguminous Seeds,‡ I have reported the results of experiments conducted by aid of an electrical criterion distinguishing between the living and not-living state.

The present communication contains the results of a series of systematic observations on the hen's egg by aid of the same distinguishing test—or blaze reaction, as I was led to designate it when it first came under my observation in the case of the frog's eyeball.

The case of the hen's egg is particularly interesting, for while we cannot tell *a priori* with any assurance whether or no a dormant egg will give the reaction characteristic of living matter, we may—after having learned by experience that it does *not* do so—expect to find the reaction make its appearance with the progress of development by incubation. And as a matter of fact, we find that this is what happens.

I first tested several eggs of unknown origin, bringing electrodes into contact with the superior and inferior extremities of a vertical short diameter of the egg laid upon its side. On account of the resistance of the shell, a small piece was removed on each side, and the electrodes brought into contact with the subjacent unbroken shell-membrane. There was no blaze in either direction, but only slight polarisation counter-currents of 0·0001 to 0·0002 volt. A similar result was obtained when the yolk was laid bare and the superior electrode applied directly to the cicatricula, both with the egg at ordinary room-temperature (20°) and in an incubator at 37°.

I then proceeded to test a series of ten fresh eggs reputed "fertile" and fit for incubation.

The results were briefly as follows:—

Egg No. 1, at the end of 24 hours' incubation, gave a small ascending blaze.

Egg No. 2, at the end of 48 hours, gave a similar but rather more distinct effect.

* "On the Blaze-currents of the Frog's Eyeball," 'Phil. Trans.,' B, vol. 194, 1901.

† "On Skin Currents. Part I. The Frog's Skin," 'Roy. Soc. Proc.,' June 6, 1901; "Part II. Observations on Cats," *ibid.*, November 21, 1901; "Part III. The Human Skin," *ibid.*, May, 1902.

‡ "An Attempt to Estimate the Vitality of Seeds by an Electrical Method," 'Roy. Soc. Proc.,' vol. 68, p. 79.

Egg No. 3, also at the end of 48 hours, gave large effects in both directions, larger and more persistent in the upward than in the downward direction. The difference between the reactions of these two eggs was in obvious correlation with their unequal degree of development, for whereas in No. 2 an area vasculosa was only just apparent at one border, in No. 3 it was well formed, and the heart was observed pulsating for more than 12 hours after exposure of the blastoderm.

No. 4 (72 hours) reacted well in both directions, and was normal.

No. 5 (72 hours) gave me pause. In spite of repeated trial I could not obtain a trace of the reaction that I expected to obtain. Every stimulus of whatever strength and direction gave rise to a slight counter-effect. But the explanation of the result was forthcoming when the egg was opened. No development whatever had taken place.

No. 6 (72 hours) gave normal reaction in both directions. Development was normal.

No. 7 (96 hours), normal reactions and normal development. Reaction abolished by rise of temperature with embryo exposed.

No. 8 (108 hours), normal reactions and normal development. Reaction abolished by rise of temperature.

No. 9 (144 hours), normal reactions and normal development. Reaction abolished by injection of a 2·7 per 100 solution of mercuric bichloride.

No. 10 (12th day of incubation) gave no blaze in either direction, only polarisation. The contents of the egg were rotten.

The above series of results was evidently in accordance with the fundamental fact. There was no exception to rule in any of the ten trials.

Certain of these ten observations were taken in closer detail in order to get at information as to relation between stimulus and response, effect of strong electrical stimulation, &c., and although these matters will demand considerable further investigation, some of the results may be described now.

This first series, by no means satisfactory from a chicken-farmer's point of view, was practically conclusive for my purpose, which was to learn whether the presence or absence of a living embryo could be diagnosed from the presence or absence of blaze-currents.

Other trials made at times of year still more unfavourable as regards probability of development, viz., in August and in November, gave results that were equally satisfactory from my point of view.

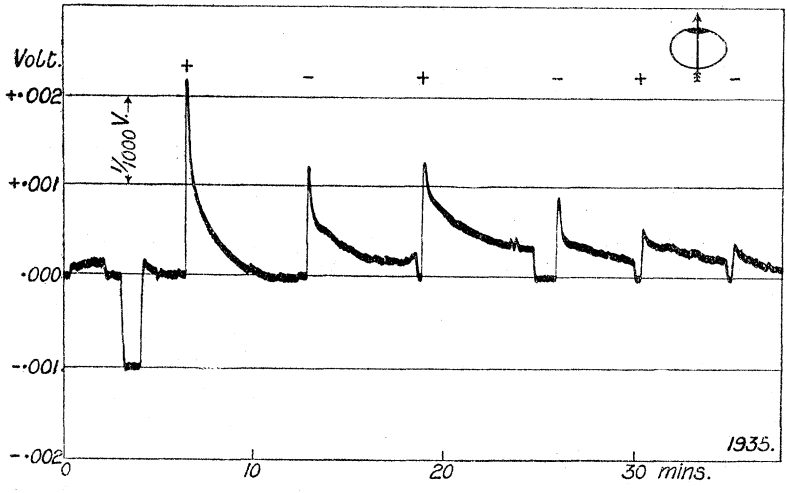
Thus in the second series, No. 1 after 32 hours' incubation gave small blaze effects of ± 0·0010 volt in both directions; the blastoderm was the size of a sixpence, and there was no sign of circulation. Nos. 2

and 3 were used in the absence of incubation for a careful examination of the exposed blastoderms, one at normal temperature, the other at 38°; polarisation currents were alone observable in these two cases, amounting to ± 0·0002 volt. Nos. 4, 5, and 6 at 50 and 58 hours gave positive blaze of only 0·0030 and 0·0010 volt, and their blastoderms were found to be very defective. Nos. 6, 7, 8, 9, and 10 gave no blaze whatever, only the usual small counter-deflections of ± 0·0002 volt due to polarisation, and on being opened exhibited no sign of development.

Incidentally to this first series of trials I noted that:—

1. The "normal current," from a developing egg, led off as described, is positive or ascending, and if the egg is left undisturbed diminishes during observation. I consider it to be a "manipulation blaze" due to handling of the egg and disturbance of the embryo. It is presumably the current first pointed out by Hermann and von Gendre in their statement that the embryo is positive to any other part of the egg contents, *i.e.*, that there is a current from content to embryo.*

Fig. 1.



Hen's Egg after 48 hours' incubation. Coil at 0. Single break shocks in ascending (+) and descending (−) directions. Response only in ascending (+) direction.

2. The embryo is easily exhausted and killed by repeated excitations of moderate and of excessive strength. Fig. 1 (No. 1935) exhibits the fatigue decline of a succession of reactions to strong induction

* Hermann u. von Gendre, "Ueber eine Electromotorische Eigenschaft des bebrüten Hühnereies," 'Pflüger's Archiv,' 1885.

shocks at intervals of 5 or 6 minutes. By strong tetanisation the reaction is completely abolished; the chick has been "electrocuted."

In a third series of ten trials upon "fresh eggs" from a London shop, and at a very unfavourable time of year as regards development, the results were as clear as could be desired. At the end of 12 days of incubation nine of these eggs returned what was by this time familiar to me as a negative answer, viz., small counter-currents due to polarisation, and all the nine showed no sign of development; only a single egg gave blaze-currents of $\pm 0{\cdot}0010$ volt, and was found to have developed to the extent usual at the end of 24 hours under ordinarily favourable conditions.

In the following year (1902) I returned to the subject and made two further series of observations, paying particular attention to the direction of blaze-currents of the first few days of incubation. In the interval between these observations and those of the previous year I had studied the currents of mucous membranes, with the general, but by no means invariable, result that the blaze-currents are of ingoing direction. I therefore expected to find, and did find, that the response of an early blastoderm to either direction of current is of positive or ascending direction, *i.e.*, ingoing as regards the hypoblast, and outgoing as regards the epiblast.

I also took the opportunity of testing active eggs on what has been described in previous papers* as the A B C plan, *i.e.*, after excitation through A, the superior pole, and B, the inferior pole of the egg, the lead-off to the galvanometer through A C was found to be effective (outgoing current at A), and the lead-off through B C ineffective. The response was outgoing or positive at A after both directions of excitation between A B.

The method of observation is further illustrated by the following table and plates. Plates 1939–40 taken on the 6th egg (72 hours) are given in detail to show how the magnitude of response varies with

1939–40. Chick Embryo. 72 hours.

Arithmetic Increase of Quantity and of Energy by increasing Capacity at Constant Voltage.

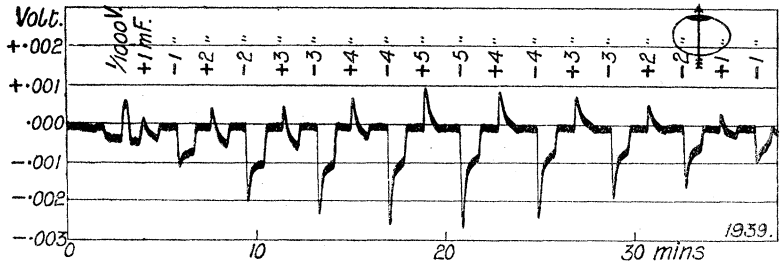
Capacity.	Pressure.	Quantity.	Energy.	Response.
1 mf.	8·4 volts.	8·4 mc.	360 ergs.	0·0010 volt.
2 ,,	8·4 ,,	16·8 ,,	720 ,,	0·0018 ,,
3 ,,	8·4 ,,	25·2 ,,	1080 ,,	0·0022 ,,
4 ,,	8·4 ,,	33·6 ,,	1440 ,,	0·0025 ,,
5 ,,	8·4 ,,	42·0 ,,	1800 ,,	0·0027 ,,

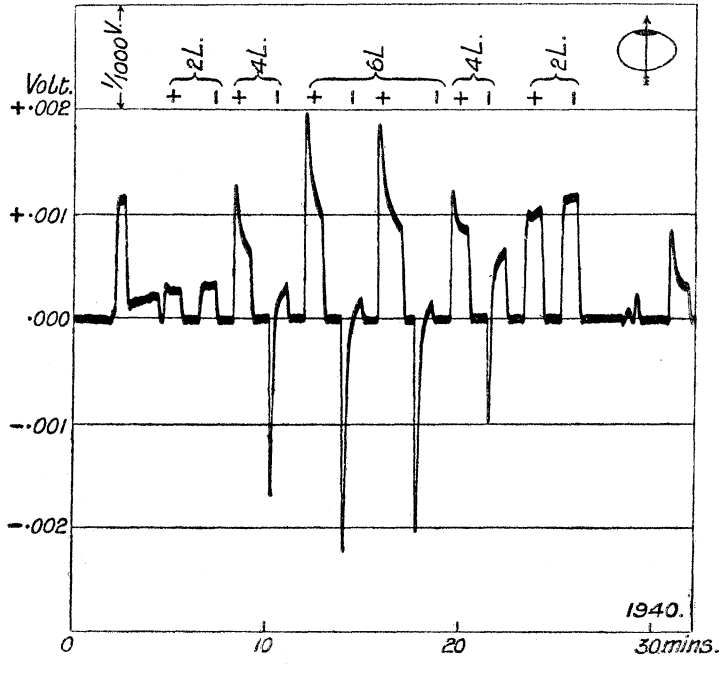
* Roy. Soc. Proc.,' vol. 68, p. 488; vol. 69, p. 181.

the magnitude of its exciting cause. The response appears to depend on energy rather than on quantity of electrical stimulus; plate 1948 taken on the 8th egg (108 hours) shows this point even more clearly. The same chick was used for examination of the influence of temperature.

Arithmetic Increase of Quantity and Geometric Increase of Energy by increasing Voltage at Constant Capacity.

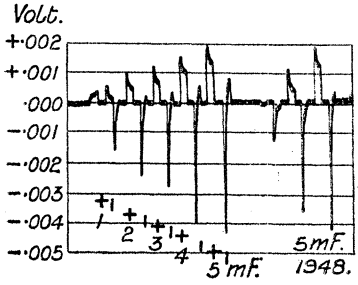
5 mf.	2·8 volts.	14 mc.	200 ergs.	0·0005 volt.
5 ,,	5·6 ,,	28 ,,	800 ,,	0·0011 ,,
5 ,,	8·4 ,,	42 ,,	1800 ,,	0·0013 ,,





Chick, 108 hours.

Pressure.	Capacity.	Quantity.	Energy.	Response.
6 L.	1 mf. +	8·4 mc.	360 ergs.	+0·0005
,,	,, —	..	..	−0·0016
,,	2 mf. +	16·8	720	+0·0009
,,	,, —	..	..	−0·0025
,,	3 mf. +	25·2	1080	+0·0011
,,	,, —	..	..	−0·0032
,,	4 mf. +	33·6	1440	+0·0015
,,	,, —	..		−0·0040
,,	5 mf. +	42·0	1800	+0·0019
,,	,, —	..	..	−0·0043
2 L.	5 mf. +	14·0	200	+0·0000
,,	,, —	..	..	−0·0015
4 L.	,, +	28·0	800	+0·0010
,,	,, —	..	..	−0·0036
6 L.	,, +	42·0	1800	+0·0019
,,	,, —	..	..	−0·0042



July 25. Chick, 108 hours.

Influence of Temperature.

Exc. by Condenser discharge. 5·6 volts; 4 L 5 mf. +

Time.	Temp.	Blaze.	Resistance.
0	28°	+0017	50,000 ω
5	28	0016	—
10	34	0014	—
15	38·5	0009	—
20	41·5	0004	35,000
25	44°	0000	—
35	37	0000	—
45	31	0000	35,000

Summary.

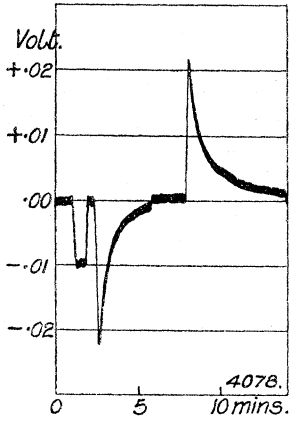
The presence of a blaze-current is a certain sign that development has progressed within the egg.

In the early stages—when presumably the blastodermic membrane has not yet become folded to form a tubular embryo—the blaze-currents aroused by both directions of excitation are positive or ascending.

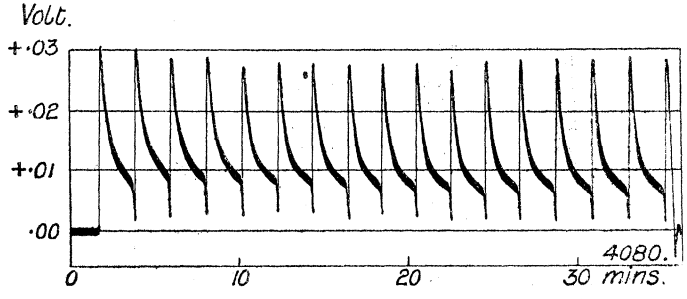
At a more advanced stage of development the blaze-currents are usually homodrome with the direction of excitation, viz., positive or ascending after a positive or ascending excitation, and negative or descending after a negative or descending excitation.

In some cases, but with such infrequency as to deserve to be characterised as exceptional, both responses have been observed to occur in a negative or descending direction. This may have been due to attachment of the embryo to the shell.

[I have made a few observations on frog's spawn, and although these have not yet been sufficient to enable me to specify the conditions of the presence or absence of the reaction, I think that the fact of its presence is worth reporting, as well as the further fact that in some cases of undoubtedly living spawn I have failed to detect it.]



Frog's Spawn.—Two homodrome responses of + and − 0·022 volt to excitation by break current at + and − 5000.



Frog's Spawn.—Series of homodrome responses to series of single break induction currents 5000 + at intervals of two minutes.

Fourth Series. June 1902. Berne Coil. 2 Leclanché's. 10,000 units.

			Single break induction shocks.		Tetanising currents.		State found on opening the egg.
			br. +	br. −	m − br +	m + br −	
1st egg	Shell broken	46 hours ..	+0·0020	+0·0010	+0·0055	+0·0030	Normal.
2nd ,,	,, unbroken	46 ,, ..	−0·0030	+0·0010	+0·0030	+0·0025	Normal.
	,, broken	46 ,, ..	+0·0025	+0·0014	+0·0040	+0·0015	Normal.
3rd ,,	,, ,,	46 ,, ..	+0·0003	+0·0002	+0·0006	+0·0005	Normal. (Strong tetanisation before test was made.)
4th ,,	,, unbroken	46 ,, ..	−0·0007	−0·0002	—	—	Doubtful result.
	,, broken	..	+0·0012	+0·0005	+0·0040	+0·0022	Normal.
5th ,,	,, ,,	66 ,, ..	+0·0025	+0·0015	+0·0090	+0·0045	Normal. Cirra vasculosa well-developed.
6th ,,	,, ,,	90 ,, ..	+0·0040	+0·0010	+0·0120	+0·0035	Normal.
7th ,,	,, unbroken	4th day ..	+0·0022	+0·0010	+0·0100	+0·0006	Strong tetanisation (8 Lecl. 10,000).
	Same egg	8th ,, ..	trace −	trace +	+0·0005	−0·0005	Embryo dead and smelling—killed presumably by strong tetanisation.
8th ,,	Shell broken	5th ,, ..	+0·0030	+0·0007	+0·0125	+0·0050	Normal.
	Egg boiled	..	−0·0002	+0·0024			
9th ,,	Shell unbroken	6th ,, ..	+0·0003	+0·0001	+0·0010	+0·0000	Doubtful result.
	,, broken	..	? trace	? trace	? trace	? trace	No sign of development. Bad smell.
10th ,,	,, ,,	7th ,, ..	+0·0008	−0·0002	+0·0030	+0·0020	Normal.
11th ,,	,, ,,	9th ,, ..	nil	nil	nil	nil	Embryo dead and smelling.
12th ,,		10th ,, ..	−0·0010	−0·0015	−0·0030	−0·0060	Well developed chick. All responses in descending direction. All responses of both eyes were positive, +0·0010 to +0·0015.
13th ,,	,, ,,	3rd ,, ..	nil	nil	trace +	trace −	No development.
14th ,,	,, ,,	3rd ,, ..	+0·0060	+0·0018	+0·0060	+0·0020	Normal.
15th ,,	,, ,,	3rd ,, ..	+0·0006	+0·0002	+0·0016	+0·0002	Normal.
16th ,,	,, ,,	5th ,, ..	nil	nil	nil	nil	No development. Bad smell. N.B. These eggs had not been turned or kept moist in the incubator for 3 or 4 days.
17th ,,	,, ,,	5th ,, ..	nil	nil	nil	nil	
18th ,,	,, ,,	5th ,, ..	nil	nil	nil	nil	
19th ,,	,, ,,	5th ,, ..	nil	nil	nil	nil	
20th ,,	,, ,,	5th ,, ..	nil	nil	nil	nil	
			The numbers in these four columns signify voltage of response.				

First Series (1901).

			Excitation.	Response.	
Unrec.	July 19	Hen's egg (from shop).....	6 L., 5 mf. ±	nil ..	Only polarisation counter-currents in both directions. Same with exposed yolk.
	,, ,,		Br O ±	nil ..	
	,, 22	1st egg (24 hours incubated)	Br O + ...	small + ..	Small ascending blaze.
	,, ,,		Br O −	nil ..	
	,, 23	2nd egg (48 hours)	Br O + ...	small + ..	Scarcely developed vascular area. Small ascending blaze.
	,, ,,		Br O −	nil ..	
1934	,, ,,	3rd egg (48 hours)	Br O + ...	+0·0010 volt ..	Well-developed vascular area. Pulsating cardiac speck. Ascending blaze.
	,, ,,	,, ...	Br O −	+0·0006 ,, ..	
1935	,, ,,	,, (60 hours)	Br O + ...	+0·0022 ,, ..	Pulsation still visible. 12 hours later. Ascending blaze.
	,, ,,	,, ...	Br O −	+0·0011 ,, ..	
1936	,, ,,	,, ...	Br O −	+0·0005 ,, ..	4 hours later. Pulsation just visible.
	,, ,,	,, ...	Br O + ...	+0·0010 ,, ..	
Unrec.	,, 24	4th egg (72 hours)	Br O + ...	+ large	Response in both directions. Well-developed embryo.
	,, ,,	,, ...	Br O −	− large	
,,	,, ,,	5th egg (72 hours)	6 L., 5 mf. +	− small	No development. No blaze. Only polarisation.
	,, ,,	,, ...	6 L., 5 mf. −	+ small	
	,, ,,	,, ...	Br O + ...	− small	
	,, ,,	,, ...	Br O −	+ small	
1939	,, ,,	6th egg (72 hours)	6 L., 5 mf. +	+0·0010 volt ..	Good vascular area and embryo. (*In extenso.*) Ascending and descending blaze.
	,, ,,	,, ...	6 L., 5 mf. −	−0·0026 ,, ..	
1940	,, ,,	,, ...	6 L., 5 mf. +	+0·0019 ,, ..	
	,, ,,	,, ...	6 L., 5 mf. −	−0·0023 ,, ..	
1941	,, 25	7th egg (96 hours)	6 L., 5 mf. +	+0·0028 ,, ..	Good embryo. Ascending and descending blaze.
	,, ,,	,, ...	6 L., 5 mf. −	−0·0044 ,, ..	Temperature raised to 40°. Reactions abolished.
1942	,, ,,	,, ...	6 L., 5 mf. +	+0·0015 ,, ..	
	,, ,,	,, ...	6 L., 5 mf. −	−0·0045 ,, ..	
1943	,, ,,	,, ...	6 L., 5 mf. +	nil ..	
	,, ,,	,, ...	6 L., 5 mf. −	nil ..	
1948	,, ,,	8th egg (108 hours)	4 L., 5 mf. +	+0·0010	Ascending and descending blaze. (*In extenso.*)
	,, ,,	,, ...	4 L., 5 mf. −	−0·0036	
Unrec.	,, ,,	,, ...	4 L., 5 mf. +	nil ..	Temperature raised to 44°. Reaction abolished.
	,, ,,	,, ...	4 L., 5 mf. −	nil ..	

1949	„ 27	9th egg (144 hours)	6 L., 5 mf. +	+0·0025	Ascending and descending blaze before $HgCl_2$.
	„ „	„	6 L., 5 mf. −	−0·0050	
Unrec.	„ „	„	6 L., 5 mf. +	nil ..	After injection of $HgCl_2$ sol. (N/10) abolished.
„	„ „	„	6 L., 5 mf. −	nil ..	
„	„ „	„			
„	Aug. 2..	10th egg. 12th day.....	6 L., 5 mf. +	− small	Bad egg. No blaze. Only polarisation.
			6 L., 5 mf. −	+ small	
			Br O + ...	− small	
			Br O −	+ small	

Second Series (1901).

„	„ 3..	1st egg (32 hours incubated)			
			6 L., 1 mf. +	+ small	Blastoderm size of sixpence. No vascular area.
			6 L., 1 mf. −	− small	
1976	—	„ „ „	6 L., 1 mf. +	+0·0012	Ascending and descending blaze before tetanisation.
			6 L., 1 mf. −	+0·0008	
1977	—	„ „ „	6 L., 1 mf. +	+0·0040	No further response after strong tetanisation.
			6 L., 1 mf. −	−0·0007	
Unrec.	—	2nd egg, fertile but not incubated	6 L., 5 mf. +	−0·0002 (about)	Blastoderm exposed. Only polarisation. No blaze.
			6 L., 5 mf. −	+0·0002 „	
„	—	3rd egg, do. do., and warmed to 37°	6 L., 5 mf. +	−0·0002 „	Blastoderm exposed. Only polarisation. No blaze.
			6 L., 5 mf. −	+0·0002 „	
	—	4th egg (49½ hours)	Br O + ...	+0·0030 „	Only ascending blaze. Area vasculosa distinct.
			Br O −	+0·0010 „	
„	„ 5..	5th egg (58 hours)	Br O + ...	+0·0002 „	Only ascending blaze.
			Br O −	+0·0001 „	
			Br O + ...	+0·0010 „	Normal current ascending + 0·0080 volt. Only ascending blaze. Area vasculosa distinct.
			Br O −	+0·0002 „	
„	„ „..	6th egg (59 hours)	6 L., 5 mf. ±	± nil	
			Br O + ...	+0·0012	Only ascending blaze.
			Br O −	+0·0001	
			Br O + ...	+0·0015	Only ascending blaze. Normal current was + 0·0035 (ascending).
			Br O −	+0·0002	
	„ 9..	7th egg (125 hours)	6 L., 5 mf. ±	nil (polarisn. ±)	No development whatever.
			Br O ±	nil (polarisn. ±)	No development whatever. R = 26,500 ω.
	„ „..	8th egg (90 hours)	Br O ±	nil	
		9th egg (90 hours)	Br O ±	nil (polarisn. ±)	No development whatever.
		10th egg (90 hours)	Br O ±	nil (polarisn. ±)	R through shell = 1,000,000, falling to 400,000 ω.

The symbol Br O signifies a break shock with secondary coil pushed home over primary.