

EFFECT OF TEMPERATURE ON THE SPORULATION AND MORTALITY OF COCCIDIAN OOCYSTS.

By MUKUNDAMURARI CHAKRAVARTY and AMIYA BHUSAN KAR, *Department of Zoology, University of Calcutta.*

(Communicated by Dr. B. Prashad, O.B.E., D.Sc., F.N.I.)

(Received August 6, 1945.)

INTRODUCTION.

A number of workers made experiments on the effect of temperature on the coccidian oocysts (Perard, 1925; Becker and Crouch, 1931; Avery, 1942) while others studied the effect of temperature on the oocysts after changing the diet of the host (Chang, 1937) or subjecting the oocysts to ultraviolet or X-Rays (Litver, 1938; Fish, 1931).

Becker and Crouch (1931) studied the effect of temperature on the oocysts of *Eimeria perforans* (Leuckart) and *Eimeria magna* Perard. Very recently Avery (1942) pointed out the effect of moderately low temperatures on the sporulation of *Eimeria debliccki* Douwes and *Eimeria scabra* Henry. The present paper deals with a similar study on the sporulation as well as mortality of the oocysts of two species of avian coccidia, *Isospora ginginiana* Chakravarty and Kar and *Eimeria pfeifferi* (Labbe).

MATERIAL AND METHODS.

The oocysts of *I. ginginiana* and *E. pfeifferi* were collected from the fresh faecal droppings of the birds *Acridotheres ginginianus* and *Columba livia intermedia* respectively. The collected oocysts were transferred in several small sporulating dishes containing 2.5 per cent potassium bichromate solution. The dishes were then kept at different temperatures maintained for the purpose.

For different temperatures the following were used, water bath at 60°C., water bath at 44°C., incubator at 37°C., and refrigerators at 22.2°C. and 4°C. Some of the sporulating dishes containing oocysts kept at room temperature varying from 28.9° to 35.5°C. served as control. The experiments were carried during the month of May, 1944, in the Zoological Laboratory, University of Calcutta.

The percentages of sporulation as well as of mortality of the oocysts of the coccidians were recorded by counting 100 oocysts every 24 hours. The data recorded in this paper represent the mean of three individual readings in each case.

The effect of different temperatures on the sporulation of I. ginginiana and E. pfeifferi.

The oocysts of this coccidian failed to sporulate at 60°C., 44°C., 37°C. and 4°C. On the other hand, cent per cent oocysts sporulated at 22.2°C. in 144 hours, while in control the maximum number of sporulation observed was 57.5 per cent in 72 hours, and at same temperature the percentage came down to 40 in 144 hours (Table I and fig. 1).

Some of the oocysts kept at 4°C. were transferred after 48 hours to room temperature and these sporulated to 58 per cent after 24 hours. The oocysts from the stocks at 60°C., 44°C. and °C. treated similarly showed negative results.

E. pfeifferi.

The oocysts of this coccidian also failed to sporulate at 60°C., 44°C., 37°C. and 4°C. Like the other species, sporulation of these oocysts occurred at 22.2°C. and at room temperature. The time required for cent per cent sporulation of the oocysts of this parasite at 22.2°C. was 96 hours, while in control only 66 per cent sporulated at the same time and the percentage came down to 28.7 in 144 hours (Table I and fig. 1).

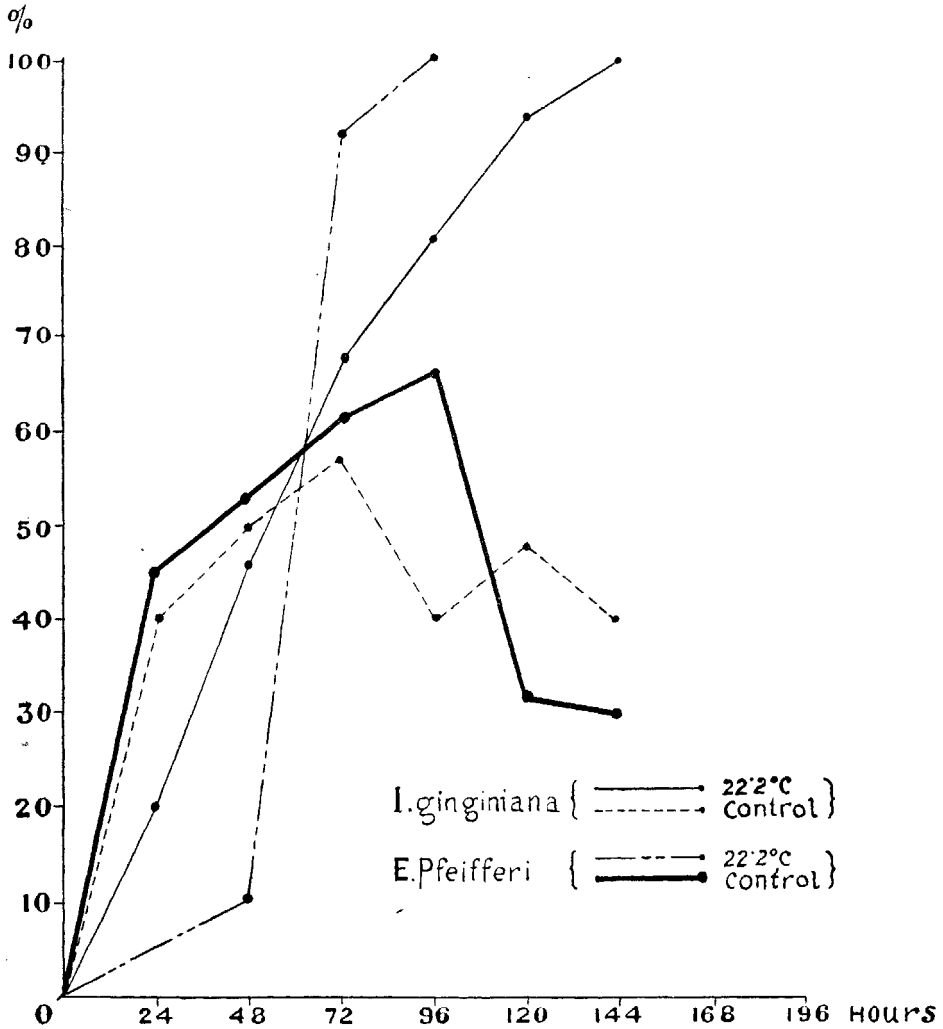


FIG. 1. Graph showing percentages of sporulation of the oocysts of *I. ginginiana* and *E. pfeifferi* at 22.2°C. and control.

TABLE I.
Sporulation percentages of the oocysts of *I. ginginiana* and *E. pfeifferi*.

Time hours.	<i>I. ginginiana</i> .		<i>E. pfeifferi</i> .	
	Temperatures.		Temperatures.	
	22.2°C.	Control.	22.2°C.	Control.
	Percentages of sporulation.		Percentages of sporulation.	
24	20.0	39.7	5.3	44.3
48	45.0	49.0	10.0	52.3
72	67.5	57.5	92.0	60.7
96	80.0	40.0	100.0	66.0
120	94.0	48.3	..	31.7
144	100.0	40.0	..	28.7

In this coccidian also no sporulation was observed when oocysts at 60°C., 44°C. and 37°C. were transferred after 24 hours at room temperature though kept there for indefinite period.

On the other hand, the oocysts kept at 4°C. for 24, 48, 72, 96, 120, 144 and 168 hours showed interesting results on being removed at room temperature for 24 hours as is shown in Table II.

TABLE II.

Percentage of sporulation of the oocysts of *E. pfeifferi* after their removal to room temperature from 4°C.

Oocyst at 4°C. for hours.	Transferred to room temperature for hours.	Percentage of sporulation.
24	24	69.3
48	"	60.0
72	"	50.0
96	"	40.0
120	"	20.0
144	"	10.0
168	"	nil

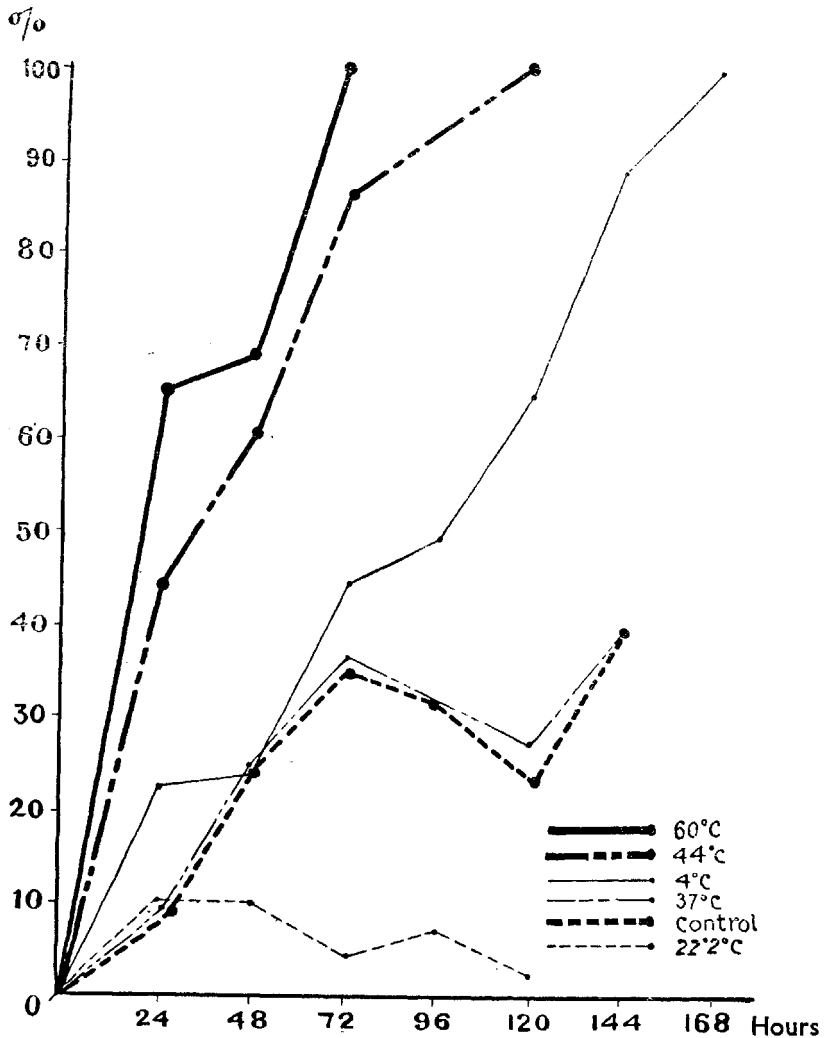


FIG. 2. Graph showing percentages of mortality of the oocysts of *I. ginginiana* at different temperatures.

From the above experiments we come to the following conclusions:—

(i) Table I demonstrates that 22.2°C. is a favourable temperature for sporulation of the oocysts of *I. ginginiana* and *E. pfeifferi*, as they sporulated to maximum number in minimum time at that temperature.

(ii) Table II shows that the oocysts of *E. pfeifferi* survived for six days at 4°C., although no sporulation occurred at that temperature but when they were transferred to room temperature, they sporulated. The percentage of sporulation, however, is inversely proportional to the subjection of oocysts at 4°C. It should be noted in this connection that the oocysts of *I. ginginiana*¹ also survived at 4°C., but when they were transferred to room temperature, they sporulated.

(iii) The oocysts of both the species did not sporulate when they were subjected to 37°C., 44°C. and 60°C. for 24 hours or a longer period.

The effect of different temperatures on the mortality of the oocysts of I. ginginiana and E. pfeifferi.

The same series of temperature employed in the preceding experiment were also used conveniently to study the rate of mortality of the oocysts of these coccidians. The percentage of mortality at room temperature served as control. The dead and live oocysts were determined by studying the zygotes within them. In a living oocyst the zygote was compact and appeared brilliant under reflected light, whereas in a dead one it became macerated and took the colour of the surrounding medium.

I. ginginiana. At 60°C. the oocysts of this coccidian were destroyed very rapidly and cent per cent death occurred in 72 hours, whereas only 5 per cent of the oocysts degenerated at 22.2°C. during this period. In control, as well as at 37°C.,² 40 per cent oocysts were destroyed in 144 hours. The time required for cent per cent kill at 44°C. and 4°C. was 120 hours and 168 hours respectively (Table III, fig. 2).

E. pfeifferi. Only 4.3 per cent oocyst of this coccidian were killed at 22.2°C. in 72 hours. The time required for cent per cent kill at 60°C., 44°C., 37°C. and 4°C. was 48, 72, 144 and 192 hours respectively. In control, however, 55 per cent of the oocysts degenerated in 144 hours (Table III, fig. 3).

TABLE III.

Mortality percentages of the oocysts of I. ginginiana and E. pfeifferi at different temperatures.

Time hours.	<i>I. ginginiana.</i>						<i>E. pfeifferi.</i>					
	Temperatures.						Temperatures.					
	4°C.	22.2°C.	37°C.	44°C.	60°C.	Control.	4°C.	22.2°C.	37°C.	44°C.	60°C.	Control.
	Percentages of mortality.						Percentages of mortality.					
24	22.0	10.0	9.5	44.0	65.0	9.5	14.3	1.6	26.7	37.0	51.3	11.0
48	24.0	10.0	25.0	60.0	69.5	25.0	10.3	2.0	26.3	68.3	100.0	15.6
72	44.6	5.0	36.5	86.0	100.0	34.5	19.3	4.3	55.6	100.0	..	19.6
96	49.3	7.5	31.5	94.3	..	32.5	19.0	..	74.6	25.0
120	64.3	2.5	27.5	100.0	..	22.5	36.6	..	86.6	40.0
144	89.3	..	40.0	40.0	56.6	..	100.0	55.0
168	100.0	85.0
192	100.0

¹ Full records of sporulation of the oocysts of this coccidian after their removal to room temperature from 4°C. were not kept.

² Further record in this temperature was not kept.

It is evident from the foregoing table that the percentage of mortality increases in higher temperature and it differs in the two species *I. ginginiana* and *E. pfeifferi* at same temperature for equal length of time.

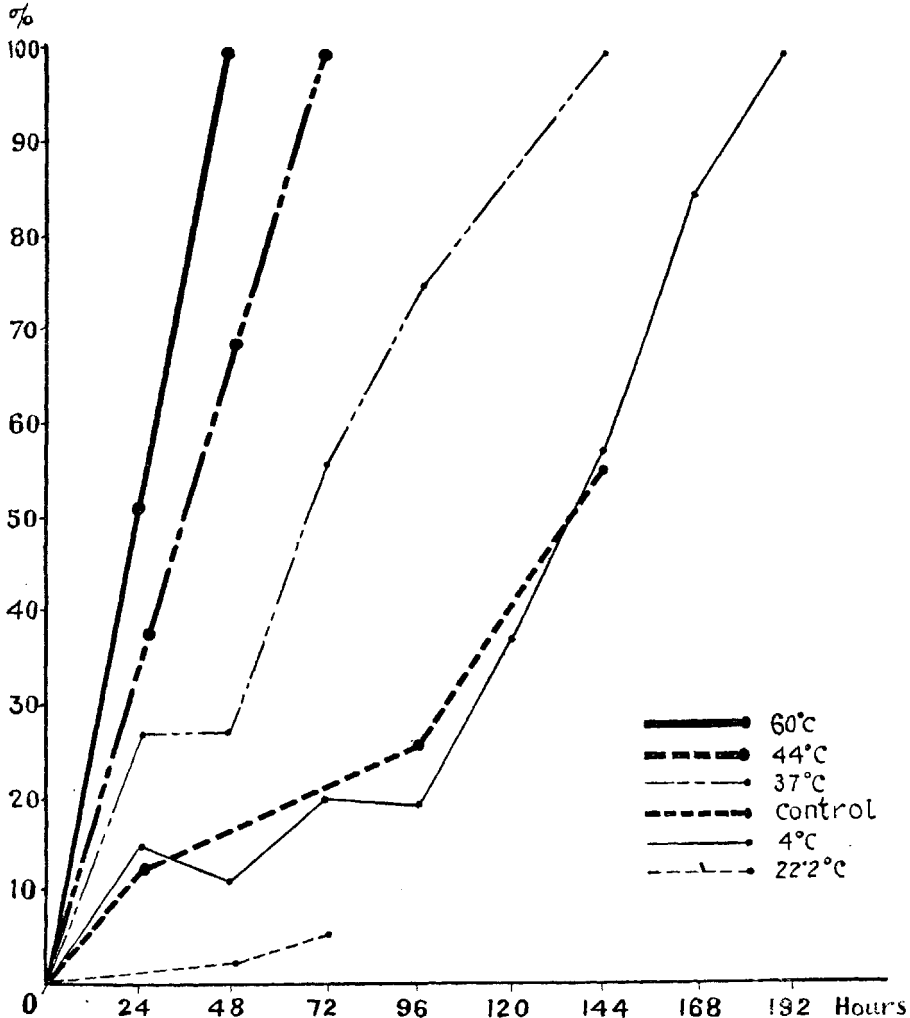


FIG. 3. Graph showing percentages of mortality of the oocysts of *E. pfeifferi* at different temperatures.

DISCUSSION.

Becker and Crouch have shown that 33°C. is the optimal temperature for sporulation of the oocysts of *E. perforans* and *E. magna*; cent per cent oocysts of the former sporulated in 48 hours while 80 per cent of the latter in 72 hours. But our observations of the same phenomenon on *I. ginginiana* and *E. pfeifferi* record an optimal height at 22.2°C.; time for sporulation of cent per cent oocysts being 144 hours for the former and 96 hours for the latter.

The thermal death point of the oocysts of the coccidians under report is as low as 37°C., for, the oocysts at that temperature for 24 hours lose the capacity to sporulate. Chang in his control experiment, found that the oocysts of *E. nieschulzi* Dieben failed to sporulate at room temperature approximating 40°C., but sporulation occurred in an incubator at 28° to 30°C. Becker and Crouch, however, observed that only 10 per cent oocysts of *E. perforans* developed at 36°C., while all of *E. magna* degenerated at that temperature.

Becker and Crouch have recorded 30 per cent oocysts of *E. perforans* developing at 10°C., while at 12°C. none of *E. magna* sporulated. Avery has reported that oocysts of *E. deblieki* and

E. scabra did not sporulate at low temperatures varying from -7°C . to 8°C . within 26 days, but when transferred to room temperature (19°C . to 26°C .), they sporulated after 14 days. Our observations on the oocysts of *E. pfeifferi* show that they can survive only for 6 days at 4°C . and if not transferred to favourable temperature within this period, they degenerate. Further the percentage of sporulation of the oocysts diminishes directly with the number of hours of their subjection at 4°C . The oocysts of *I. ginginiana* also can sporulate if transferred to room temperature from 4°C . The results of Avery and those obtained here show that low temperatures are not fatal for further development of the coccidian oocysts for a number of days.

Fish stated, 'The time required to kill washed, unsegmented oocysts is inversely proportional to the degree of heat used'. Chang, on the other hand, has shown that unsporulated oocysts of different species of *Eimeria* do not respond to heat in the same manner when treated under identical laboratory conditions. Both the statements hold good for *I. ginginiana* and *E. pfeifferi* as the percentage of mortality of the oocysts is increased with the rise of temperature and the time required for cent per cent mortality at different temperatures varies to some extent in the two species.

It is seen from the above experiments that there is a definite co-relation between the sporulation and mortality percentages of the coccidian oocysts, as for instance, the percentages of sporulation of the oocysts of *I. ginginiana* and *E. pfeifferi* at 22.2°C . are indirectly proportional to the percentages of mortality at that temperature.

From the above facts, mainly two questions might arise—(1) Why do the oocysts of the coccidia *I. ginginiana* and *E. pfeifferi* sporulate at a lower temperature than those of *E. perforans* and *E. magna*? (2) Why do the oocysts of *I. ginginiana* and *E. pfeifferi* degenerate after 6 days at 4°C ., while those of *E. deblicieki* and *E. scabra*, as shown by Avery, can stand a still lower temperature for a period of 26 days and then when transferred to room temperature, they sporulate after 14 days?

As to the first question, it is evident that though the coccidians under investigation belong to different genera, yet they behave similarly in the sporulation of the oocysts. Becker and Crouch have also observed the same behaviour of two different species of *Eimeria*. It appears, therefore, that the sporulation of the oocysts at different temperatures is not wholly dependent on their specific difference. The difference might as well be due to differences to their hosts which belong to two separate classes, viz., Aves and Mammalia.

As to the second question, it seems probable that the oocysts of *E. deblicieki* and *E. scabra* are more adapted to lower temperature than those of *I. ginginiana* and *E. pfeifferi*, as the oocysts of the former two species are adapted to low climatic temperature in nature. It might be pointed out that further work on this common coccidian *E. pfeifferi* in other countries may throw some light on this point.

SUMMARY.

1. Cent per cent oocysts of *I. ginginiana* and *E. pfeifferi* sporulated at 22.2°C . and the percentage of sporulation is higher than in control.
2. The oocysts of these coccidians do not develop at 37°C ., which can therefore be regarded as the thermal death point of the oocysts.
3. A low temperature as 4°C . is not fatal for further development of the oocysts which in case of *E. pfeifferi* survive for 6 days at this temperature. If the oocysts are transferred to favourable temperature within this period, they sporulate.
4. The mortality of the oocysts increases with the rise of temperature and the time required for cent per cent mortality differs in the two species, *I. ginginiana* and *E. pfeifferi*.

REFERENCES.

- Avery, J. L. (1942). The effect of moderately low temperatures on the sporulation of oocysts of two species of Swine Coccidia. *Jour. Parasit.*, **28** (Supplement), 28.
- Becker, E. R. and Crouch, H. B. (1931). Some effect of temperature upon development of the oocysts of Coccidia. *Proc. Soc. Exp. Biol. and Med.*, **28**, 529-530.
- Chang, K. (1937). Effects of temperature on the oocysts of various species of Eimeria (Coccidia, Protozoa). *Amer. Jour. Hyg.*, **26**, 337-351.
- Fish, F. (1931). The effect of physical and chemical agents of the oocysts of *Eimeria tenella*. *Science*, **73**, 292-293.
- Litver, G. M. (1938). Susceptibility of the sporogony process in oocysts of coccidia to ultra-violet rays as dependent upon temperature conditions and the reparative power of the oocysts. *C.R. Acad. Sci., U.R.S.S.*, **20**, 693-696.
- Perard, C. (1925). Recherches sur les coccidies et les coccidioses du lapin. II. contribution a l'étude de la biologie des oocysten des coccidies. *Ann. Inst. Past., Paris*, **39**, 505-542.