

which are considered conventionally non-hosts for arbuscular mycorrhizal fungi (AMF) which interact with almost 90% of the terrestrial plants<sup>21–23</sup>. However, only limited members of the plant community have failed to interact; these belong to the families of Amaranthaceae, Chenopodiaceae, Cyperaceae, Juncaceae, Proteaceae or with lupines and Cruciferae, etc.<sup>22–26</sup>. A careful perusal of the literature indicates that this statement may not be completely true<sup>27,28</sup>. Denison *et al.*<sup>26</sup> have emphasized that model systems are also important as a new research tool to understand the cooperation between microbes and the plants. Members of these families, including the model plant *Arabidopsis thaliana* lack symbiotic interactions such as mycorrhizae and rhizobia. The present study emphasized an interaction of endosymbiotic fungus *P. indica* with most members of cruciferae tested including *A. thaliana*<sup>7</sup>. This study further strengthens earlier results<sup>18</sup> that *P. indica* interacts with most plant groups and does not discriminate the members of Cruciferae, but fails to interact with myc<sup>−</sup> mutants (these also failed to interact with AMF)<sup>10</sup>.

This observation opens up an approach for application of plant-promoting symbiotic fungus *P. indica* for better production of crops of agricultural and horticultural importance. It also provides models to understand the interaction between plants and plant-microbes at the molecular level.

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## Larval case renovation – a unique behaviour in bagworm moth, *Eumeta crameri* Westwood

Bagworms are a group of highly specialized lepidopterans belonging to the family Psychidae and exhibit extreme development of sexual dimorphism. Males are winged whereas females lack functional appendages. Larvae of both males and

females, soon after hatching from the eggs climb up to the top of their host plants in order to have an access to the soft and palatable tips of the growing shoots. They construct a small but tough bag of silk of either cylindrical or conical

shape and glue small fragments of plant tissues around their cases. Larvae always keep their body inside the cases. While moving about, their head and thorax are protruded out so that they move forward on their thoracic legs dragging the case

behind them, which is gripped by hooks on the abdominal prolegs. When taking rest, the rims of the cases remain attached to a twig by means of silken thread so that the cases hang vertically with both their openings remaining closed. The characteristic sexual dimorphism, fascinating gait and peculiar case architecture collectively make each individual bagworm a biological curiosity.

The bagworm larva grows in size until the room inside its case becomes insufficient to accommodate it. Eventually it expands the size of its case by discarding the older materials, such as thorns and/or twigs and attaches newer and bigger materials. Here, this phenomenon is referred to as case-renovation behaviour. In the present study an attempt has been made to ascertain the exact number of larval instars and obtain information on case-renovation behaviour and its periodicity in *Eumeta crameri*.

The life cycle of bagworm moth, *E. crameri* Westwood was studied using newly hatched larvae harvested from the host plant, *Acacia nilotica* only. Saplings of *A. nilotica* were raised specifically for the purpose.

Study-I commenced on 30 June 1996. Five synchronously hatched bagworm larvae were plucked from the host plant, *A. nilotica* and were kept inside wire-meshed cages. The larvae were provided with cut-twigs from *A. nilotica* daily. Water was sprinkled twice everyday to maintain freshness of the leaves and twigs. Observations were recorded daily on number of case renovations, length of the longest stick, and dates of gluing of the first and the last sticks on each episode of case renovation during the entire period of larval development and growth till all of them entered into the pupal stage. Each larva was maintained individually inside a cage. The total number of days taken for the emergence of adults was noted.

Study-II also commenced on 30 June 1996, but in the natural habitat of the bagworms. Twenty bagworm larvae were numbered by putting nail-polish marks of various colours in different combinations for counting and identifying the larvae (from JN # 01 to JN # 20). Observations were recorded daily on the number of case renovations, length of the longest stick, and dates of gluing of the first and the last sticks on each episode of case renovation during the entire period of larval development. Five larval cases were

plucked when they had silken bags only around their body. Body weight, larval length and head capsule width were recorded. Five new cases were harvested soon after the completion of each bout of case renovation, i.e. the first, second and third renovations. The above-mentioned end points were monitored.

Study-III was a mere repetition of study-I. However, in the present case the study commenced on 30 September 1996. Ten synchronously hatched larvae were harvested from *A. nilotica* plants and were reared in the laboratory till all of them entered the pupal stage. The end points mentioned in study-I were monitored.

Study-IV commenced on 30 September 1996. Ten synchronously hatched bagworm larvae were numbered using nail-polish of different colours. Observations were made on all the larvae in their natural habitat. Variables mentioned under study-I were monitored.

Means and standard errors were computed for various morphometric variables and comparison between means was performed by Duncan's multiple-range test.

Correlation and regression coefficients were computed for pairs of variables from among number of instars, length of the longest stick, body weight, age, larval length and head capsule width. Except number of instars and age, all other variables were log-transformed.

Life history of bagworm moths as gauged from larval growth and development was studied rigorously under four different sets; two under captive conditions in the laboratory and the remaining two in their natural habitat. Two sets consisting of one under laboratory conditions and another under natural habitat belonged to a generation that occurred in the month of June and the other two sets belonged to a different generation initiated in the month of September. Figure 1 illustrates the life history of *E. crameri*.

For study-I under laboratory conditions, bagworm larvae undertook case renovation three times before their entry into the pupal stage (Table 1). During each episode of case renovation the larvae used longer sticks, progressively (Table 1). The time lag for the completion of case renovation was the least during the third renovation. On an average, the larvae took 61.2 and 91.4 days for entry into the pupal stage and for emergence of adults respectively. Among the five adults that emerged, three were males.

The data obtained under study-II were heterogeneous. The data gathered for larvae before the first case renovation and after the first, second and the third case renovations do not belong to the same individual organism. On each instar mentioned above, five larvae were plucked. They were removed from their bags and subjected to morphometry. Thus, in this study data on larval length, larval body weight and cranium width were obtained (Table 2), in addition to those on number of case renovations, length of the longest stick, and dates of gluing of the first stick and the last stick. The larvae as reported in study-I used longer sticks progressively during the course of their growth and development (Table 1). The larvae completed case-renovation work much more quickly during the last renovation compared with the first renovation. Both larval length, body weight and head capsule width increased progressively during the course of their growth and development (Table 2).

Study-III yielded data on ten synchronously hatched larvae reared under laboratory conditions. Identical results were obtained with those reported under study-I with regard to length of the longest stick and time taken for the completion of nest renovations (Table 1). The larvae took on an average, 53.5 days for entry into the pupal stage (Table 1).

In study-IV also, larvae used statistically significant longer sticks progressively during the course of their growth and development, leading to their entry into the pupal stage. However, case-renovation was quicker during each instar compared to those observed under study-I and study-III. The larvae also took less number of days for entry into the pupal stage (Table 1).

The correlation matrix showing the relationships between variables like number of instars, larval age, body weight, length of larva and head capsule width is given in Table 3. The correlation coefficient was found to be statistically significant for all pairs of possible combinations. Regression constants for all pairs of variables are shown in Table 3. The relationship was found to be linear and statistically significant for all pairs of variables (Table 3; Figure 2a and b).

Correlation and regression coefficients were also computed for two variables, namely, number of larval instars and the size of the longest stick for each individual organism observed under study-I,

**Table 1.** Case renovations and length of the longest stick during larval development of bagworm moth

LLS (mm)	First renovation		Second renovation		Third renovation		TLP (days)	TLA	CS
	TLC (days)	LLS (mm)	TLC (days)	LLS (mm)	TLC (days)	LLS (mm)			
Laboratory condition: ( <i>N</i> = 5)									
9.8 ± 0.2*	7 ± 0	11.8 ± 0.37	5.2 ± 0.8	21.8 ± 1.52	3.8 ± 0.73	33.2 ± 1.24	61.2 ± 1.74	91.4 ± 1.74	2 females and 3 males
Natural condition: ( <i>N</i> = 5)									
10.2 ± 0.45	5 ± 1.09	19 ± 0.54	2 ± 0.77	26.4 ± 1.77	1.8 ± 0.8	34 ± 2.50	—	—	—
Laboratory condition: ( <i>N</i> = 10)									
10.3 ± 0.26	3.2 ± 0.8	17.4 ± 0.65	2.3 ± 0.63	25.6 ± 0.83	1.3 ± 0.15	33.7 ± 1.36	53.5 ± 1.33	—	—
Natural condition: ( <i>N</i> = 10)									
9.7 ± 0.3	2.2 ± 0.2	12.4 ± 0.16	1.6 ± 0.4	19.3 ± 0.49	1.9 ± 0.48	38.1 ± 1.45	31.6 ± 1.34	—	—

\*Mean ± SE of data obtained from laboratory and natural conditions. LLS, Length of the longest stick; TLC, Time lag for completion of each episode of case renovation; TLP, Time lag for entry into pupal condition; TLA, Time lag for emergence of adult, and CS, Composition of sex.

**Table 2.** Age, body weight, larval length, head capsule width, length of the longest stick and time taken for completion of case renovation of bagworm larvae during each instar of larval development. (Mean ± SE of data obtained from studies conducted under natural conditions; n = 5 during each instar, thus N = 20)

Instar	Age	Body weight (mg)	Larval length (mm)	Head capsule (mm)
1st	5 ± 0*	17.28 ± 1.97 <sup>c</sup>	9.2 ± 0.37 <sup>d</sup>	0.91 ± 0.03 <sup>c</sup>
2nd	15.6 ± 0.93	216.58 ± 23.10 <sup>b</sup>	16 ± 1.14 <sup>c</sup>	2 ± 0 <sup>b</sup>
3rd	19.8 ± 1.2	230 ± 17.29 <sup>b</sup>	20.4 ± 0.81 <sup>b</sup>	2.4 ± 0.24 <sup>a,b</sup>
4th	29 ± 1	589.34 ± 85.93 <sup>a</sup>	26 ± 1.76 <sup>a</sup>	2.8 ± 0.2 <sup>a</sup>

\*Means bearing similar superscripts are not statistically significant from each other (based on Duncan's multiple-range test).



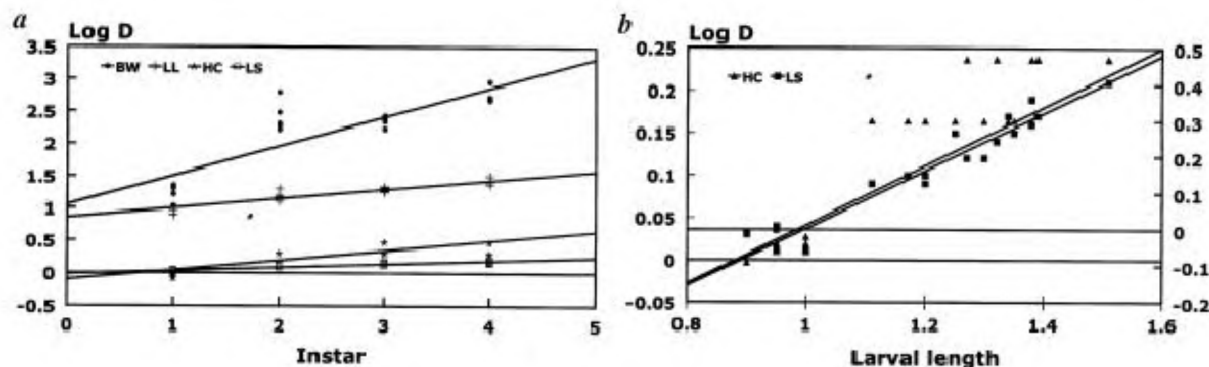
**Figure 1.** Life cycle of bagworm moth *Eumeta crameri* Westwood in host plant *Acacia nilotica*. (1) Case showing couple of ootheca; (2) First instar larva; (3) Second instar larva; (4) Third instar larva. One thorn is disproportionately longer than the others in the case; (5) Fourth instar larva. Two thorns are disproportionately longer than the others in the case; (6) Emergence of puparium indicating the time of adult emergence; (7) Adult male moth; (8) Adult female moth.

study-III and study-IV. The relationship between number of instars and length of the longest stick used by the bagworms in their cases was found to be linear and statistically significant for 22 out of 25 individual bagworms (Table 4).

The bagworm moth, *E. crameri* Westwood appears to be very common in the Indian subcontinent<sup>1,2</sup>. It has been shown to have multiple hosts, namely *Camellia thea*, *Acacia* sp., *Albizia falcata*, *Casuarina equisetifolia*, *Eucalyptus grandia*, *Gmelina arborea*, and *Psidium guajava* in southern part of India<sup>3,4</sup>. It has also been reported from Bangladesh<sup>2</sup> and has been known to infest a large number of plant species, including *Litchi chinensis*, *Thuja* sp., *Quisqualis indica*, *Rosa* sp., *Punica granatum*, *Bougainvillea* sp., *Ficus religiosa* and *Saraca indica*<sup>2</sup>. However, interestingly no serious attempts have been made to do an in-depth study of the life cycle of such a common bagworm moth species of the Indian subcon-

tinent. Ameen and Sultana<sup>2</sup> have reported its life cycle; however, they could not ascertain the number of larval instars through which *E. crameri* undertakes metamorphosis to attain adulthood. They did not comment on the phenomenon of case renovation, which is a characteristic feature of the life cycle of many bagworm species. This phenomenon has also not attracted the attention of several authors who have studied some aspect of biology of *E. crameri* Westwood<sup>3,4</sup>. The present study ascertains the exact number of larval instars and incorporates information on case renovation behaviour and case renovation cycle in *E. crameri*.

There is a fundamental problem in the study of the life cycle of any bagworm moth and to ascertain the number of larval instars it has. Soon after hatching, the young larva makes a protective silken bag (case) around its body and later on glues leaves/thorns/sticks/spines/twigs, etc. over its original case. When it moves,



**Figure 2.** *a*, Linear relationship between number of larval instars and body weight (BW) or larval length (LL) or head capsule width (HC) or length of the longest stick (LS). Data shown in Y-axis are log-transformed. *b*, Linear relationship between larval length and head capsule width (HC) or length of the longest stick (LS). Data shown in both axes are log-transformed.

**Table 3.** Correlation and regression coefficients and other relevant statistics illustrating the relationship between number of larval instars, age, body weight, larval length, head capsule width and length of the longest stick glued to the case during larval growth and development in bagworm moth, *E. crameri*

	<i>r</i>	se of <i>r</i>	<i>b</i>	se of <i>b</i>	<i>a</i>	<i>n</i> - 2	<i>P</i>
Variable: Instar							
Age	0.96	0.06	7.62	0.47	-1.7	18	< 0.001
BW	0.85	0.12	0.45	0.06	-1.9	18	< 0.001
Ll	0.93	0.08	0.14	0.01	0.8	18	< 0.001
HC	0.87	0.11	0.15	0.01	-0.1	18	< 0.001
LS	0.95	0.07	0.05	0.00	-0.0	18	< 0.001
Variable: Age							
BW	0.90	0.10	0.06	0.00	-1.8	18	< 0.001
Ll	0.94	0.08	0.01	0.00	0.9	18	< 0.001
HC	0.89	0.10	0.01	0.00	-0.0	18	< 0.001
LS	0.93	0.08	0.00	0.0005	0.0	18	< 0.001
Variable: Body weight							
Ll	0.90	0.09	0.26	0.02	1.4	18	< 0.001
HC	0.93	0.08	0.30	0.02	0.5	18	< 0.001
LS	0.89	0.10	0.09	0.01	0.2	18	< 0.001
Variable: Larval length							
HC	0.91	0.09	1.02	0.10	-0.9	18	< 0.001
LS	0.96	0.06	0.33	0.02	-0.3	18	< 0.001
Variable: Head capsule width							
LS	0.87	0.11	0.27	0.35	0.0	18	< 0.001

*r*, Correlation coefficient; se of *r*, Standard error of correlation coefficient; *b*, Slope; *a*, Intercept; *P*, probability; BW, Body weight; Ll, Larval length; HC, Head capsule width; LS, Length of the longest stick.

only the head and thorax come out of the bag and upon receiving the slightest provocation, it retreats inside the case. If the larva is taken out of its case, then the entire process of growth and development stops. Thus, all these factors taken together make it difficult for the investigators to ascertain the exact number of larval

instars and to monitor various growth-associated variables *in situ*.

The June and September studies were conducted to rule out possibilities of the effects of 'generation' on case-building behaviour. Further, the laboratory investigation was performed to assess the effects of captivity on the same behaviour.

Results of these studies reveal that the bagworms belonging to two different generations behaved alike and that under captivity, there was a marginal slowing down of the process of larval growth and development. However, the pattern of behaviour remained the same irrespective of their status, i.e. under captivity in the laboratory or free living in its natural habitat.

The phenomenon of larval case renovation involves interesting and intricate mechanisms. When the larva realizes that the space inside the case is not optimal, it cuts optimum-sized thorns or twigs and glues it to the rim of the bag at around 45° inclination. When the required number of thorns/twigs have been cut and glued, the larva bites away the wall of the bag along the length of one of the old thorns/twigs which is cut loose and eventually discarded. The new thorn/twig is pulled into its place to fill the slit and glued along the whole length with fresh silk. *E. crameri* performs case renovation three times prior to its entry into the pupal stage.

Does *E. crameri* renovate its case once between two consecutive instars? This hypothesis has been tested using size of the longest stick in its case as a tool. When a bagworm larva reaches its final size it suspends the case from a twig, closes, both anterior and posterior openings, turns its head downwards towards the bottom opening and then pupates. Prior to this it fastens its case rigidly by tying it onto a twig with several layers of silken thread. At this moment more force has to be applied to pull apart the case from the twig. In contrast, during other stages of development it is relatively easy to pull apart the case from the twig by applying slight force.

**Table 4.** Correlation and regression coefficients and other relevant statistics illustrating the relationship between number of larval instars and length of the longest stick glued to the case during larval growth and development in bagworm moth, *E. crameri*

	<i>r</i>	se of <i>r</i>	<i>b</i>	se of <i>b</i>	<i>a</i>	<i>n</i> – 2	<i>P</i>
Under laboratory conditions							
Study-I							
JL # 01	0.98	0.10	0.2	0.02	0.7	2	0.010
JL # 02	0.98	0.12	0.2	0.02	0.7	2	0.015
JL # 03	0.97	0.16	0.2	0.31	0.7	2	0.027
JL # 04	0.98	0.12	0.2	0.02	0.7	2	0.016
JL # 05	0.96	0.18	0.2	0.03	0.7	2	0.033
Study-II							
SL # 01	0.98	0.10	0.2	0.02	0.8	2	0.011
SL # 02	0.96	0.19	0.1	0.03	0.9	2	0.038
SL # 03	0.97	0.17	0.2	0.03	0.8	2	0.031
SL # 04	1.00	0.06	0.2	0.01	0.8	2	0.004
SL # 05	1.00	0.15	0.2	0.02	0.8	2	0.023
SL # 06	1.00	0.06	0.2	0.01	0.9	2	0.003
SL # 07	1.00	0.04	0.2	0.01	0.8	2	0.001
SL # 08	0.90	0.31	0.1	0.04	1.0	2	0.106 <sup>NS</sup>
SL # 09	1.00	0.07	0.2	0.01	0.8	2	0.005
SL # 10	0.96	0.19	0.1	0.03	0.9	2	0.037
<i>In situ</i> in nature							
Study-III							
SN # 01	0.97	0.15	0.2	0.03	0.7	2	0.024
SN # 02	0.97	0.17	0.2	0.03	0.7	2	0.031
SN # 03	0.97	0.17	0.2	0.04	0.7	2	0.031
SN # 04	1.00	0.09	0.1	0.02	0.7	2	0.008
SN # 05	0.98	0.15	0.1	0.02	0.8	2	0.022
SN # 06	0.94	0.24	0.2	0.05	0.7	2	0.062 <sup>NS</sup>
SN # 07	0.98	0.12	0.2	0.02	0.8	2	0.015
SN # 08	1.00	0.05	0.2	0.01	0.6	2	0.002
SN # 09	0.94	0.23	0.2	0.05	0.8	2	0.054 <sup>NS</sup>
SN # 10	0.98	0.13	0.2	0.02	0.8	2	0.019

J, June; L, Laboratory conditions; S, September; N, Nature.

Results of the present study clearly reveal that the bagworm larva takes less time to pupate while in its natural habitat than when it is reared under captive conditions. The time taken for the completion of case renovation is also shorter in its natural habitat. During the episode of the second case renovation, the larva uses longer thorns/twigs than that used during the first case renovation. Further, during the third and the final case renovation it uses still longer thorns/twigs than used during the previous renovations.

Growth in animal body is always more or less cyclic, periods of comparative rest alternating with periods of activity<sup>5</sup>. This phenomenon is much more evident in insects. In many insects, the amount of growth which is achieved at each moult/instars is predictable from certain empi-

rical laws. When the number of instar is plotted against logarithm of some measurement on the insect, a straight line is generally obtained. In the present study, a straight line is obtained not only for the variables such as body weight, larval length and cranium width based on the insect, but a straight line is obtained for the length of the longest thorn/twig used by the insect on its protective case. Results of this study corroborate many others for diverse variables in *Bombyx mori*, *Philosamia ricim*, *Cladius isomerus* and *Tenebrio molitor*<sup>5</sup>. The results of this study are close to those reported for *T. molitor*<sup>5</sup>. However, this study provides information that a variable, i.e. length of the longest stick, an inanimate object, behaves much like any other biological variable, such as body weight, larval length and head capsule width.

After hatching, *E. crameri* may metamorphose into an adult within an average time lag of 91.4 days. However, there may be several unknown limiting factors that may not allow the emergence of adults. This happened in one of the studies reported here. Adults did not emerge from the pupal cases even one year following pupation. The pupal cases were kept under close observation till the end of the year with the anticipation that the adults may emerge soon after the monsoon. However, in July all cases perished without a single emergence. Therefore, it seems that the length of diapause may vary from one generation to another of the life cycle in bagworms and adult emergence may not take place if favourable conditions are not present.

It could be concluded that *E. crameri* has at least four larval instars during its

life cycle, and it renovates its case at least once between two consecutive instars. The study of case construction<sup>6,7</sup> and its renovation in bagworms appears to be interesting.

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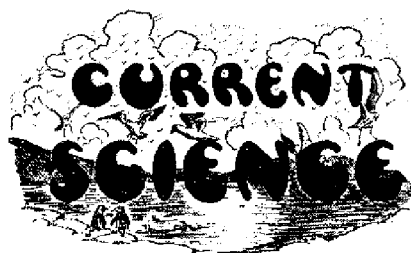
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### **The manufacture of glandular products: The organization of our slaughter houses**

The chemist in this country who attempts work on glandular products, either for large-scale production in a factory, or for preparation on a small scale in the laboratory, is brought, sooner or later, inevitably to the realization that his raw materials, namely, the various animal glands, though potentially vast in quantity, are not available to him in the manner and form in which he would have desired to obtain them. While several hundreds of animals are killed daily in the slaughter houses of the cities in India, there is at present no provision made for facilities for the preservation of these glands in order that they might be worked up later, or be transported to a distant place where the central factory for the manufacture of the glandular hormones might be situated. The organizations which have been perfected in the

abattoirs of Europe and the Packing Houses of America are, it is sad to state, conspicuous by their absence in India. Few of us are, perhaps, aware that the collection and export of these tiny glands form the basis of a flourishing trade in South America, and still fewer realize that, even apart from such American firms as Park Davis Co., and others, some of the biggest pharmaceutical concerns in Great Britain are dependent to-day mainly on the countries across the Atlantic for their supply of raw materials for the manufacture of products like insulin, pituitrin, etc. The classical researches of Harington on the structure of Thyroxine might, perhaps, never have been accomplished but for the large amounts of raw materials in the shape of Thyroid glands supplied by the South American cattle yards. One of the chief secrets of this splendid organization in Europe and America lies in the high efficiency which has been attained in the technique of large-scale refrigeration. Insulated chambers, cooled by refrigerating machinery, are the essential features of all modern abattoirs. Immediately after the animals have been killed, the dressed carcasses are removed to the cold rooms. They are kept there for several hours during which all the endocrine glands are removed neatly under expert supervision. They are then frozen and conveyed immediately in iced and insulated trucks to local firms, if there are any, or packed into large refrigerated chambers in ships which take

them all the way across the Atlantic. In our country, on the other hand there are, I believe, no slaughter houses which can boast of insulated cold rooms. The result is that there is little time available for the selection and removal of the glands which, in several cases, have their potent principles destroyed to a large extent even by an hour's delay of removal, due to the setting in of autolysis.

A word in this connection about the methods of killing animals in our abattoirs may not be out of place. Cattle and sheep are, as a rule, not stunned as is the humane practice adopted in the abattoirs of Europe and America; on the contrary, the animals are almost always subjected to the horrible sights and smells of the slaughter house, and they often witness the sufferings of their fellows. One of the most important glandular products, Adrenaline, is known to be the hormone of emergency, and it is not at all improbable that in the struggle of the animals during the few minutes immediately preceding death, a considerable portion of the stock of this hormone in their glands finds its way into the blood stream, and is thereby lost. In introducing reforms in these wasteful practices, it is necessary not only to rouse the public conscience but also to have the intelligent co-operation of some of the meat-eating communities in India in bringing about the necessary relaxation of their religious scruples.

B. B. Dey