TABLE I

Date	Cat no.	Condition before extract was given	Results
11/21/30	257	In convulsions	Improved in 30 minutes; walking about in 1 hour; eating 2 hours after injection
12/ 5/30	273	In convulsions	Improved 30 minutes after injection; in splendid condition 6 hours later
12/ 7/30	273	In convulsions	Again rapidly resuscitated following administration of extract
12/10/30	273	Comatose	Sat up 15 minutes following injection; appeared normal 1 hour later
12/11/30	273	In convulsions	Recovery from extreme prostration in 4 hours
1/28/31	276	Comatose	In splendid condition 5 hours after injection
1/30/31	276	In convulsions	Appeared normal within 3 hours; ate salmon

tose animal. We have also used the extract subcutaneously and intramuscularly in man without ill effect.

Our extracts made according to the Swingle-Pfiffner method contain from one-in-one-million to one-in-two-million parts of adrenalin. By control experiments we have shown that the recoveries are not due to adrenalin activity, although we have frequently found the adreno-medullary hormone effective in restoring severely prostrated animals to an apparently normal condition. Much larger amounts of adrenalin than are contained in the cortical extracts must however be given. The restoration with adrenalin is, furthermore, only temporary, lasting usually from 4 to 12 hours. Similarly the recovery from prostration following the injection of glucose solution is very short-lived. We are in definite disagreement with the statement of Swingle and Pfiffner[2] that adrenalectomized animals with severe symptoms show no improvement in their condition and "derive no benefit from the injections" of adrenalin.

It may be noted that the quantity of extract it is necessary to give to prostrate adrenalectomized animals to effect complete restoration represents relatively huge amounts of cortical tissue—a thousand times or more than the amount present in the normal cat. Swingle and Pfiffner, as well as ourselves, frequently gave from 15 to 32 cc of the extract, representing up to nearly 1,000 grams of the adrenal cortex, in the course of 12 hours, to resuscitate animals with severe symptoms. And the normal cat possesses only from 200 to 300 milligrams of cortico-adrenal tissue altogether! It seems likely that previous observers may not have secured noteworthy effects with their cortical extracts because of failure to employ the heroic dosage necessary.

Our experience with cortico-adrenal extracts made according to the Hartman technique is somewhat limited, because of the difficulties of preparation mentioned above. The few batches we have prepared we have tested a great number of times on adrenalectomized cats, and in some cases we have obtained indications of the presence of the hormone in the Hartman extracts.[4]

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PREMATURE REVERSAL OF HEART-BEAT IN BOMBYX

IT is now well known[1] that in Lepidoptera as in Ascidea there is a regular alternation in the direction of heart-beat, which, in insects, begins in the prepupa and continues during the pupal and adult stages. It is understood, of course, that the direction of heart-beat in most insects is forward, toward the head.

Intermittent backward beating does not normally occur in the full-grown silkworm until it has stopped feeding, evacuated its intestine, spun its cocoon and rested about 24 hours. Then, about 48 hours before pupation, periodic reversal of direction normally begins.

Yokoyama,[2] 1927, has recently stated that this change in type of circulation can be brought on prematurely by closing the posterior pairs of spiracles with enamel paint or by injecting lactic or acetic acid into the 8th abdominal segment. His published graphs apply to the full-grown silkworm during the 5th stage, though he claims to have obtained almost identical results with larvae of the 4th. To induce reversal, according to this observer, 6 pairs of spiracles must be blocked at the beginning of the 5th stage (one day after moulting), one pair less on each successive day, until during the last 5 days (including the period of spinning) only the 3 most posterior pairs (segments 6–8) need be blocked. Similar results

[4] Grateful acknowledgment is made of aid in the above investigations by the Grants-in-aid Committee of the General Education Board.

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[1] J. H. Gerould, *Jour. Morph. and Physiol.*, 48: 385–429, 1929.

[2] T. Yokoyama, *Dobutsagaku Zachi* [Zool. Mag.], 39, No. 459, Suppl.: 45–51, 1927.

were obtained by injecting lactic acid into the 8th abdominal segment, an n/3 solution being necessary at the beginning (one day after the moult) but only n/6 or n/7 after the first day.

Repeating and extending these experiments in July, 1930, I found that the methods used paralyzed the muscles of the body wall and heart and thus suspended forward beating. When recovery took place, a few backward beats ensued in some individuals, but the tendency to beat forward was so strong that in the unevacuated larvae of the 5th stage either forward beating exclusively was resumed or none at all.

In a large individual of this stage (6 cm in length), for example, the 6 posterior pairs of spiracles were blocked with Brunswick black at 5 P. M. on July 11. Forward beating continued, and at 10 A. M. the next day only forward beating was observed. At 3 P. M. a second coat of enamel was applied to the same 6 pairs of spiracles (segments 3–8) and in addition those of the 2nd abdominal segment were blocked. Circulation forward clearly continued. The muscles of the abdominal wall and prolegs were paralyzed; head and thorax were active. Twenty-four hours later (4 P. M., July 13) there were occasional pauses in forward beating but no reversal.

With older worms which had evacuated the intestine and were about to spin, blocking 3 or 6 pairs of spiracles was somewhat effective in inducing premature reversal, and, if one wraps cotton swabs about the tips of a pair of forceps, dips them in ether, strong alcohol or xylol and holds them against the last three pairs of spiracles (6–8 abdominal), periodic reversal at this stage can be readily induced. Backward beating was more distinct under these conditions than when the spiracles were closed with Brunswick black. A phase of 12 backward beats was counted in one case. In another (after ether), a backward phase continued for 30 minutes, but, as soon as the effect of anesthesia disappeared, exclusively forward beating was restored. Xylol was applied to the 3 most posterior spiracles in a spinning larva: a short pause in peristalsis quickly ensued and then 162 backward beats were observed. Afterwards the heart beat was forward persistently and an attempt to reverse the direction by applying alcohol to the 5th segment (segments 6–10 being paralyzed) failed.

These experiments indicate a strong neuromuscular metabolic gradient from the posterior end of the dorsal vessel. That such control is localized in a ganglion within the walls of the posterior end of the vessel is made improbable by the following experiment upon the individual in which the long-continued backward phase (30 minutes) had been induced by etherizing the posterior segments. The 6th abdominal segment was tightly constricted by a strong thread and, five hours later, the posterior end of the body (abdominal segments 7–10) was amputated. Forward beating without reversal was observed for over a half hour. Evidently forward peristalsis is produced by no special sympathetic ganglion at the posterior end. Nor is the presence of the ventral nerve cord necessary, for peristalsis continues when the dorsal body wall is excised with the heart upon it.

The injection of an aqueous suspension of carmine through the caudal horn in a larva ready to spin was followed by a phase of vigorous backward beating, after only feeble backward beats had been induced in this individual by etherizing the last 3 pairs of spiracles. The impingement of the irritating fluid upon the aorta, with its now heightened irritability, produced a reflex like the action of an emetic.

Yokoyama found that immersion of the rear end of a mature larva in water induced premature reversal. Repeating this experiment, the 3 posterior pairs of spiracles being immersed, I observed, after peristalsis had stopped and recovery was beginning, a few feeble backward beats. Six were counted after one immersion, upon complete recovery from which, constant forward beating was reestablished. To one individual a series of 3 immersions was given, after two of which a few backward beats were observed.

Immersion in 95 per cent. alcohol covering the last 3 pairs of spiracles was more effective. Nine backward beats of more pronounced character were counted in one case.

It has just been shown that amputation of the posterior segments (7–10, abdominal) does not interfere with normal forward peristalsis of the dorsal vessel in the larva, and the heart upon the excised dorsum of the adult reverses periodically.[3] It would be expected, accordingly, that the amputated abdomen of the pupa would show periodic reversal. Such was found to be the case, as the following experiment shows.

A slip-noose of strong thread was drawn tightly around the 1st abdominal segment of a soft pupa immediately after shedding the larval skin, and the head and thorax were clipped off. Central beating or double action (a form of backward beating) occurred in the abdominal heart before and during this operation. Holding the squirming abdomen after a few minutes between thumb and fingers to control the intense muscular action, it was seen that completely backward phases from the 2nd abdominal segment were alternating with forward phases. Another isolated abdomen prepared in this way was kept alive 24 hours after amputation and 42 hours after con-

[3] *Jour. Morph. and Physiol.*, 48: 422, 1929.

striction and virtual separation from the thorax. Normal periodic reversal was observed several times throughout this period. It is clear that head and thorax are not necessary to initiate backward beating of the dorsal vessel.

Since Japanese lacquers are different from the American, it may be possible that the enamel paint used by Yokoyama would have a different effect from that produced by our Brunswick black and so induce backward beating in the less mature larva. It should be noted, however, that local paralysis in the less mature larva brought on by the use of Brunswick black often did not check forward beating. The insect apparently had not reached the stage of development at which backward beating was possible; so that, upon partial recovery from the operation, only forward pulsation occurred. In the more mature larva ready to spin, narcotization of the posterior segments, if not intense, still inhibits very imperfectly the strong tendency to beat forward.

That minute quantities of acid added to Ringer's solution quickly paralyze the dorsal vessel of the larva was shown by Pigorini,[4] 1917, who found that acetic acid at a dilution of $\frac{1}{1000}$ and formic acid at even greater dilution ($\frac{1}{5000}$) were instantly lethal.

To solve the problem of backward beating one should first answer the fundamental question, why it beats forward. Evidently there is a pronounced metabolic gradient from the posterior end of the larva which is never lost and only intermittently neutralized. To it, as development proceeds, is added the condition in which the middle region (young pupa) or the aorta (adult) are intermittently more active. These gradients are adaptations to the great influx of hemolymph (1) into the posterior end of the heart, (2) into the region of the node of double-action or central beating in the young pupa (usually between abdominal segments 3–4), and (3) from the thorax, with its pulsating mesothoracic vesicle, in the older pupa and adult.

SUMMARY

(1) Premature reversal of direction of heart-beat in *Bombyx* was induced in spinning larvae and those about to spin by blocking the 3 most posterior pairs of spiracles with Brunswick black, in accordance with the experiments of Yokoyama.

(2) This method and the injection of lactic and other acids paralyze the muscles of the body wall and heart in that region, thus preventing forward and permitting backward beating.

(3) Attempts to produce periodic reversal in the larva in the early days of the 5th stage by Yokoyama's methods failed.

(4) The application of ether, alcohol, or xylol to the 3 posterior pairs of spiracles, or immersion of these segments in alcohol, was effective in inducing premature reversal at the close of the 5th stage, exclusively forward beating being resumed as soon as narcosis disappeared.

(5) Amputation of the posterior end of the body of the larva, including the end of the dorsal vessel in abdominal segments 7 and 8, did not prevent forward peristalsis.

(6) Amputation of head and thorax from the abdomen of a pupa did not interfere with normal periodic reversal in the abdomen.

(7) Thus periodic reversal in the dorsal vessel takes place without the intervention of any terminal ganglia.

(8) The suggestion of Yokoyama and earlier writers (Bataillon, Fischer) that general acidosis initiates normal backward beating is not corroborated.

(9) The metabolic gradient of the larval dorsal vessel is never lost, but intermittently neutralized in the prepupa, pupa and adult by increased metabolic action at two other points: the central node (in the young pupa) and the aorta with its mesothoracic pulsating vesicle (in the adult).

(10) These gradients are adaptations to the large influx of hemolymph into the dorsal vessel at three principal regions.

The writer gratefully acknowledges assistance rendered by a grant from the Joseph Henry Fund. Useful suggestions by Dr. C. C. Stewart were incorporated in this article during its revision.

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[4] Atti. R. Acc. Lincei, Anno 314, Ser. 5, Vol. 26, 2° Semest., p. 15–19, 1917.

AMOEBOID MOTION AS THE PRODUCT OF PROTEIN SWELLING

THE nature of protoplasmic movement, as seen in its simplest form in the extrusion of pseudopodia by the amoeba, is still a matter of debate. Whether we deal with the apparently haphazard actions of the free-swimming amoeba or the directional movement of a leucocyte, the fundamental nature of and the reason for the movement remain obscure. The most popularly accepted belief is that the amoeba suffers a surface tension change, but the material in which such surface tension change occurs has never been touched upon to my knowledge. It occurred to me that the extrusion of a pseudopodium by an amoeba might depend upon a localized swelling with consequent softening of a surface area in a properly "stim-