TWO TYPES OF RHYTHMIC MOVEMENTS OF CHICK EMBRYOS IN THE AMNIOTIC FLUID

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Investigation of rhythmic movements of the chick embryo showed that, besides movements with a frequency of 6-20/min due to contractions of the amnion (5th-14th days of incubation), slower movements with a frequency of 1-3/min also occur. These slower movements are also observed after the 5th day, and they continue until the yolk sac is fully drawn in before hatching. Differences in the action of muscle relaxants suggest that the movements of the 1st and 2nd types are of different origins, and that those of the second type result from contractions of the skeletal muscles of the embryo itself.

During development of the chick embryo, not only active movements caused by the muscles of the embryo itself, but also passive movements resulting from contraction of the amniotic sac are observed.

Rhythmic contractions of the amnion are observed from the 5th to the 14th days of incubation with a frequency of 6-20/min [1, 4, 7, 8]. Contractions of the amnion have been observed oscillographically in embryos as early as the 4th day [6]. After the 14th day of incubation, contractions of the amnion cease because of the disintegration and gradual degeneration of its muscular layer [5, 9].

Observations in the writer's laboratory have shown that, starting from the 5th day of embryonic development, rhythmic passive movements occur not only at a frequency of 6-20/min, but also at a much lower frequency. It has been suggested that these movements are associated with other muscular structures. The object of this investigation was to examine the nature and source of these contractions.

EXPERIMENTAL METHOD

Passive movements were recorded by converting the movements of the membranes into electrical oscillations which were recorded on a loop oscillograph [2].

Chick embryos of the White Leghorn breed were used in the experiments. The eggs were incubated at 38° in a laboratory incubator.

Movements of the membranes were observed and recorded in an acute experiment in eggs with the shell and shell membranes opened from the side of the air chamber. On the 14th day of incubation the embryo, together with the yolk sac, was poured from the egg into a bath containing physiological saline; its connection with the allantois, which remained in the sheli, was preserved.

Contractions of the amnion were observed and recorded from the 5th to the 14th days, and movements of the yolk sac from the 6th to the 19th days. Electrodes of the detector for recording movements were fixed to the wall of the amnion or to the vitelline membrane.

EXPERIMENTAL RESULTS

When the electrodes were located on the amnion, two different types of contractions were distinctly observed. The first of these, with a frequency of 6-20/min, was observed from the 5th until the 14th day of development. The frequency of these contractions varied with age: it increased from the 5th to the 8th day

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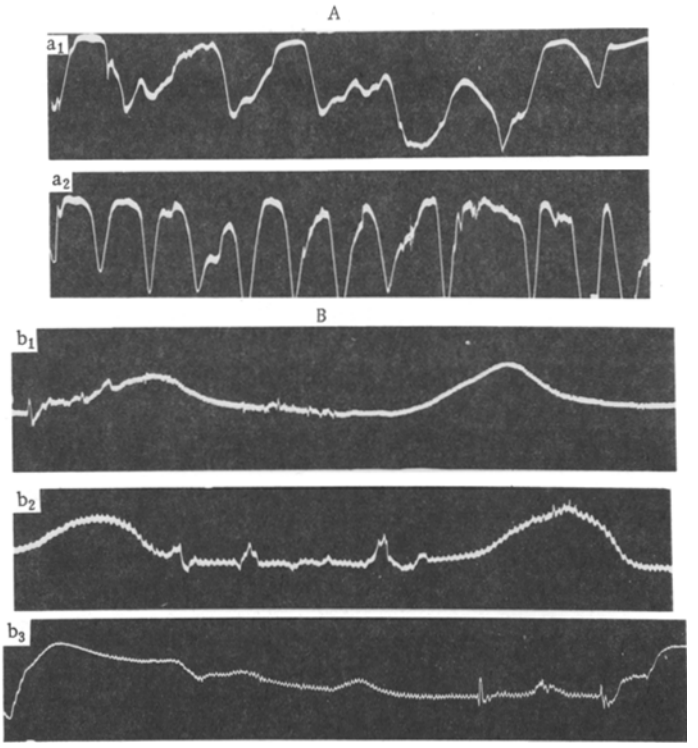

Fig. 1. Spontaneous rhythmic movements. A) Movements of amnion (a_1, age 7 days; a_2, aged 10 days); B) movements of yolk sac (b_1, age 6 days; amnion not injured; b_2, age 8 days, amnion destroyed; b_3, age 17 days, amnion not functioning).

and decreased from the 9th to the 14th day. Contractions of this frequency have also been observed by other workers both in the intact chick embryo [1] and in an isolated strip of amnion [4, 7, 8]. The writer's visual observations, together with graphic recordings, also indicate that movements of this type are associated with contractions of the amnion (Fig. 1A).

Contractions of the second type (Fig. 1B) were found from the 5th day of incubation, and their frequency did not exceed 1-3/min. The duration of each contraction varied from 12 to 15 sec, whereas the duration of each contraction of the first type was 2-7 sec. No other investigator has reported the existence of a rhythm of this type. These contractions showed no significant change from the 6th to the 19th day of incubation, and they remained after surgical destruction of the amnion (in the first half of incubation), and after its degeneration (in the second half of incubation), indicating their independence of contractions of the amnion. Visual observations showed that contractions of this type are associated with movement of the yolk sac.

Published data relating to movements of the yolk sac are concerned only with the period of hatching of the chick, when this sac is drawn inside the body cavity of the embryo (19th-20th day of incubation). As Rogazina [3] states, from the 17th day of incubation the yolk sac begins to be overgrown by its muscular layer, which is derived from the abdominal wall of the embryo. She associates the mechanism of indrawing of the yolk sac into the abdominal cavity with contractions of these muscles. Lillie [9], on the other hand, ascribes this process entirely to contractions of the embryo's abdominal wall, and does not mention overgrowth of the yolk sac by the muscular layer. Although these observations apply only to the period of hatching, they do suggest that the skeletal muscles of the embryo play a part in producing the movements of the yolk sac which were recorded in the present experiments starting from the 5th day of development. Contractions of the amnion, on the other hand, as many investigators have firmly established, are produced by nerveless smooth muscle fibers.

TABLE 1. Doses of Muscle Relaxants

Day of incubation	D-tubocurarine		Succinylcholine		Method of administration of drug
	concn.	dose (in ml)	concn.	dose (in ml)	
6—7th	1:10 000	0,1	1:10 000	0,1	Application to surface of amnion
8—11th	1:1 000	0,1—0,2	1:1 000	0,1—0,2	
12—15th	1:10 000	0,05—0,1	1:10 000	0.005—0,1	Injection into body of embryo
16—19th	1:1 000	0,1—0,2	1:1 000	0,005—0,1	

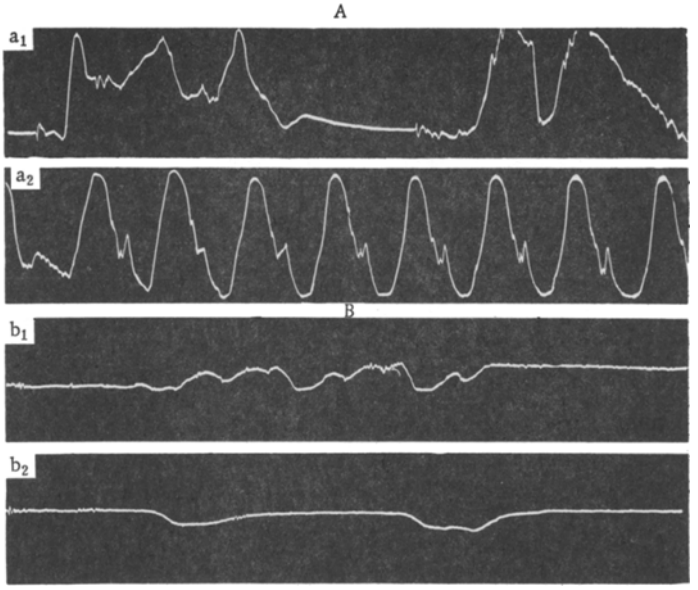

Fig. 2. Action of tubocurarine on movements of amnion (A) and yolk sac (B) (age 12 and 15 days, respectively). a_1, b_1) Before administration of tubocurarine; a_2, b_2) 2 min after administration.

It can be concluded that different muscle tissues are concerned in the formation of these two types of movements: smooth nerveless muscles of the amnion in the first case, and skeletal muscles of the embryo in the second case. If this hypothesis is correct, these structures must differ in their relationship to muscle relaxants. To verify this deduction, a series of experiments was carried out to study the action of D-tubocurarine and succinylcholine on the course of rhythmic contractions of the first and second types.

Solutions of D-tubocurarine and succinylcholine, made up in physiological saline, were used in different concentrations depending on the time of incubation. Minimal doses stopping movements of the embryo were chosen. From the 6th to the 11th days of development, the drug was applied directly to the amniotic membrane; from the 12th day the drug was injected subcutaneously into the embryo's thigh or wing (Table 1).

The experiments showed that tubocurarine in most cases causes activation of amniotic contractions, as shown by an increase in the duration of the periods of activity and increased frequency and regularity of the rhythm of contractions (Fig. 2A). In the early stages of development (from the 6th to the 9th days of incubation), tubocurarine also activates movements of the yolk sac, while in the later stages of development (15th-19th days of incubation) tubocurarine has the opposite effect: it reduces the frequency of the contractions or inhibits them completely (Fig. 2b).

Succinylcholine also differed in its action on the contractions of the first and second types. During the action of succinylcholine on the amnion, inhibition (an increase in the duration of the rest periods) or total cessation of the movements was observed in 71% of cases. The inhibitory effect was observed

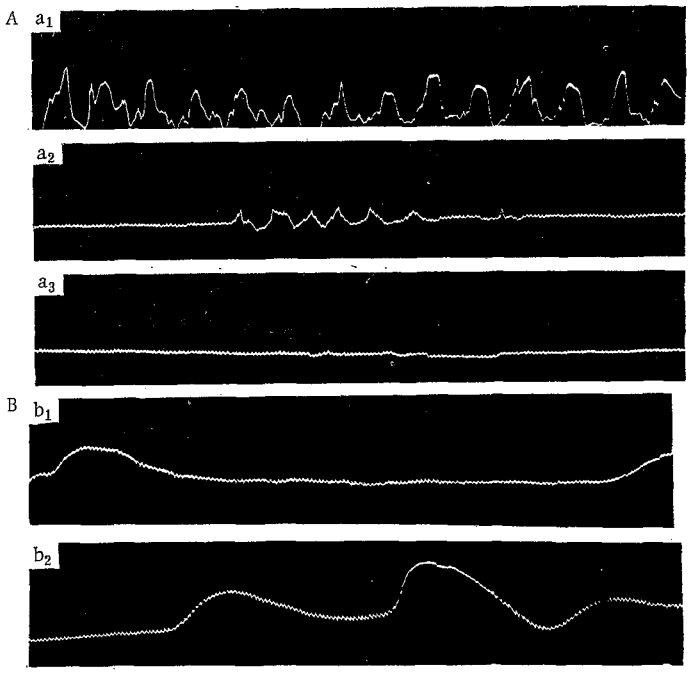

Fig. 3. Action of succinylcholine on movements of amnion (A) and yolk sac (B) (age 8 and 9 days, respectively). a_1, b_1) Before administration of succinylcholine; a_2, b_2) 2 min after administration; a_3) 4 min after administration.

throughout the period of activity of the amnion – from the 6th to the 13th day (Fig. 3A), and activation of amniotic contractions was observed only in 2% of the experiments. The action of succinylcholine on movements of the yolk sac was expressed either as inhibition or as activation through the period of embryonic development. However, activation of movements predominated throughout the period of incubation (activation in 58%, inhibition in 36% of the experiments). In some experiments (6%) no action of succinylcholine could be observed (Fig. 3B).

Control observations showed that physiological saline, in which the solutions of tubocurarine and succinylcholine were made up, itself can inhibit contractions of the membranes. It might be concluded from analysis of the results that the effects of physiological saline and of succinylcholine were similar. However, physiological saline acts on contractions of the amnion only during the first minutes after its administration, after which the normal contractions are restored, while the effect of succinylcholine becomes stronger with the passage of time. The time of physiological saline is always weaker than that of succinylcholine.

After administration of succinylcholine the response of the yolk sac, like that of the amnion, was more permanent and definite in character than its response to injection of physiological saline.

It can be concluded from these results as a whole that by the 5th day of embryonic development of the chick, both the amnion and yolk sac participate in its passive movements. However, movements of the embryo relative to the amniotic fluid can be regarded as passive only if they are produced by contractions of the walls of the amnion, which are known to possess their own pacemaker mechanism [6, 7]. Movements of the embryo connected with movements of the yolk sac, on the other hand, are not passive in the true meaning of the term, for they are evidently associated with the skeletal muscles of the embryo itself. The results of the experiments with muscle relaxants described above are evidence in support of the view that these movements are connected with the skeletal muscles of the embryo.

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