sium chloride, histamine, etc.(7,10,11,12). Therefore, either one must postulate some different mechanism for pain mediation or these polypeptides are only one of a number of mediators with perhaps special activity in endothelial or mesothelial-lined structures.

Summary. In man, the polypeptides bradykinin and kallidin, but not eledoisin, produce pain on intraperitoneal injection. None of these substances produces pain when injected subcutaneously or intramuscularly in higher concentrations. Therefore, none of these polypeptides can be considered as a universal mediator of pain.

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Heart Rate of the Developing Chick Embryo.* (32490)

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The two common techniques used to measure heart rate of avian embryos require removing a portion of the shell to form an observation window(1,3) or implanting electrodes within the egg(4-6). These procedures expose the embryo to shock, injury and infection. In the latter case there can be tissue reaction to the electrodes, which may be further aggravated by frequent embryonic movements causing changes in position of the implanted probes. Temperature also influences the frequency of embryonic heartbeat; it has been reported that 0.5°C fluctuations were sufficient to cause considerable rate changes(4). In order to determine a "normal" embryonic heart rate, a method should be employed which does not require penetration of the shell or other disturbance to the embryo and which allows maintenance of the normal incubation environment.

A miniature ballistocardiograph developed at the Ames Research Center(7,8) provides a method by which motions within a living organism can be monitored with a minimum of disturbance to it. This instrument is an ultra-sensitive piezoelectric transducer which utilizes some of the basic principles and components of a micrometeoroid momentum transducer(9), but is especially designed for specific application as a high frequency ballistocardiograph for avian embryos. The device measures the acceleration of the egg shell induced by embryonic movement, especially heart beat, and incorporates electrical and mechanical common-mode noise rejection features, The object of the present study was to compare heart rates of developing chick

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embryos obtained by ballistocardiography with those previously obtained by other methods.

Methods and materials. The average heart rate was determined each day for each of 56 Single Comb White Leghorn embryos from the 4th to the 9th day of incubation (Group a), and from the 7th day to the 19th day of incubation (Group b), when pipping and hatching begins. On the 3rd day of incubation the intensity of the heartbeat signals were weak and variable and thus were not included in this report.

The ballistocardiograph transducer was mounted on a free floating platform in a Napco bacteriological incubator maintained at a constant 37.5°C (Fig. 1). The embryos

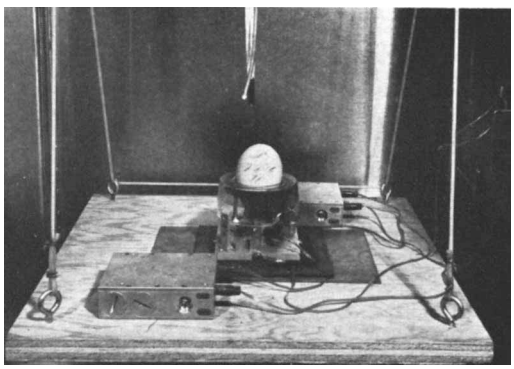


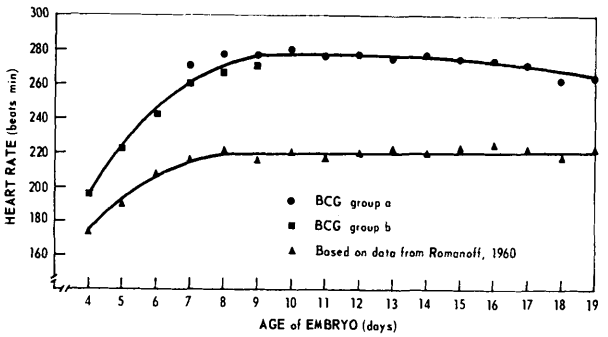
FIG. 1. Avian ballistocardiograph sensing apparatus. The piezoelectric transducer and pre amplifiers are suspended in a bacteriological incubator.

were incubated in a modified Lyons display incubator(10) situated adjacent to the ballistocardiograph. The eggs were turned through 90° every 2 hours and temperature was maintained at 37.5°C with relative humidity at 60%. Eggs were placed one at a time on the basket of the transducer in the same vertical position as when situated in the Lyons incubator. The transfer from one incubator to another required less than 10 seconds and was done without jostling which might disturb the embryo. To insure the quiescent state of the embryos, 2 to 5 minutes elapsed before the BCGs were recorded. The temperature in every instance was 37.5°C at the top of the egg during recording. The output of the piezoelectric transducer was amplified, filtered, and displayed on an oscilloscope screen as a recognizable ballistocardiogram. When the signal appeared to be free of other embryonic movements, it was recorded by an Offner RS Dynograph recorder (Beckman Instruments). Heart rate was then determined from the BCG using 12-beat and/or 2-beat segments. Embryos from the 4th to the 9th day (Group a) were monitored in an isolated chamber maintained at 37°C to prevent cooling during transfer and eliminate the majority of external environmental noise. The embryos were sexed at termination of each experiment and daily heart rate averages were computed separately for each sex.

A second group of White Leghorn eggs was used to compare the results of the ballistograph method with an electrocardiograph method. Heart rate of 18-day-old embryos was established first with the ballistocardiograph method; following this electrodes were implanted by drilling 2 small holes in the shell, and inserting fine wire (#42) through the shell membrane. The electrocardiogram (ECG) obtained in this manner was compared *simultaneously* with the ballistocardiogram (BCG) and recorded on 2 separate channels of the Offner pen recorder. The embryos were allowed several minutes to recover from handling before recording. Temperature and other environmental factors were as previously described.

In a third phase of this study, the heart rate of chick embryos from 4 different genotypes was measured, chosen because differences in heart rate had been observed in the adults. The heartbeat frequency of 40 embryos (10 per group) was compared daily from day 10 to hatching time. The genotypes used in this investigation were Single Comb White Leghorn (light breed); Australorp (heavy breed); mutants homozygous for the autosomal recessive gene Scaleless (scsc) which lack scales and most feathers(11,12); and progeny of a cross between Australorp and Scaleless (+/sc).

Results. The results of this investigation differ markedly from previous studies. The daily heart rate of 56 embryos exceeds that found by previous investigators by 50 to 70 beats per minute (Fig. 2). The maximum average heart rate (280 bpm) was reached



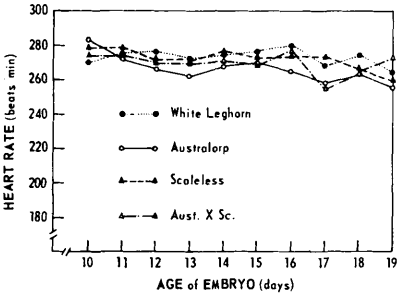


FIG. 2. Chick embryo heart rates during development. Ballistocardiography compared with previous methods.

FIG. 3. Heart rates from 4 genotypes of chick embryos.

on the tenth day of incubation and decreased slightly thereafter. This is equivalent to the heart rate reported for day-old chicks(13) and is substantially higher than reported previously for developing chick embryos. Some embryos showed considerable day-to-day variation in heart rate. In a very few cases a fluctuation of 5 or 10 beats per minute was seen over several minutes of recording from the same embryo; most embryos, however, had a consistent rate throughout incubation. The standard deviations of the group means increased toward the termination of incubation. The average daily heart rate of the 4 genotypes measured revealed no significant differences between the lines (Fig. 3). After the seventh day, the average heartbeat frequency was slower in males than in females on 12 of 13 days; this is significant at the 5% p level (Table I).

Heart rates obtained by ballistocardiography were identical to those obtained by electrocardiography when embryos were measured simultaneously with both methods. The average heartbeat frequency from BCGs made just prior to preparing the embryos with electrodes was 260.8 bpm; after penetrating the shell with probes however, the average embryonic heart rate decreased to 249.6 bpm (Table II). With some embryos there was a very pronounced decrease, while others showed only slight or no alterations in

TABLE I. Average Daily Heart Rate of Single Comb White Leghorn Chick Embryos.

	Males		Females	
Day	No. of embryos	av bpm ± S.E	No. of embryos	av bpm ± S.E
4	22	196.8 ± 1.5	16	195.9 ± 1.5
5	31	223.1 ± 1.8	25	222.8 ± 1.8
6	31	243.9 ± .9	25	243.2 ± .9
7a	31	261.3 ± 1.0	25	261.0 ± .9
7b	20	270.2 ± 2.1	16	272.1 ± 2.1
8a	31	266.7 ± .9	25	266.8 ± .9
8b	19	277.5 ± 2.5	17	278.7 ± 3.1
9a	31	268.9 ± .9	25	272.0 ± 1.2
9b	25	276.0 ± 1.6	23	278.5 ± 1.6
10	27	278.7 ± 1.3	29	282.1 ± 1.0
11	26	276.0 ± .7	28	277.3 ± 1.4
12	27	276.8 ± 1.4	29	277.9 ± 1.4
13	27	274.1 ± 1.0	29	275.3 ± 1.1
14	26	275.4 ± 1.0	28	277.3 ± 1.5
15	27	276.7 ± 1.4	29	274.6 ± 2.0
16	27	273.3 ± 1.5	29	274.1 ± 2.1
17	27	268.9 ± 2.0	29	273.8 ± 2.6
18	27	260.0 ± 2.5	29	264.6 ± 2.5
19	27	261.8 ± 2.5	28	265.0 ± 2.4

TABLE II. Comparisons of Heart Rate in Embryos Before (BCG) and After Electrode Insertion (BCG and ECG).

Individual embryo	Before electrodes BCG (bpm)	After electrodes ECG, BCG (bpm)
A	270	255
B	245	245
C	265	240
D	240	235
E	255	245
F	250	240
G	265	260
H	250	255
I	285	275
J	280	260
K	270	245
L	255	240
Mean	260.8	249.6

frequency. Embryos in the latter case usually had a heart rate slower than average for the group.

Discussion. The ballistocardiograph provides a method by which the heart rate of chick embryos can be measured without changing normal incubation conditions. There is no mechanical injury, shock, tissue-probe interaction, or possibility of infection involved with this procedure. Temperature can be very accurately controlled and maintained at the optimal level. The egg is in the normal vertical incubation position and all other environmental factors remain constant.

The heart rate of the chick embryo decreases rapidly in response to slight mechanical disturbances, injuries or cooling. Previously reported observations probably do not indicate the true activity of the developing embryonic heart due to the induced trauma. The rates obtained are lower than those recorded here and often were based on a small sample of embryos. Romanoff(14) indicates in his composite graph a variation of 40 to 80 bpm among embryos measured on the same day. Our results show a consistent range of about 20 to 30 bpm among embryos throughout most of the incubation period with a slight increase in variability occurring toward the termination of incubation.

The chick embryos used in the first two phases of this experiment are the result of crossing inbred lines of chickens and are thus genetically similar. No significant differences in heart rate are apparent between the four very different types of chick embryos used in the third phase of this investigation. Therefore, some of the variability in our measurements, and in measurements by other, is probably a response to minor alterations of the embryo's environment by handling.

Summary. A new technique was used to measure the heart rate of developing chick embryos. Ballistocardiography should afford ideal conditions for observing embryonic heartbeats with a minimum of disturbance to the embryo. The same embryos can be measured on each day of incubation. The heart rate increased from day 4 through day 10 and then decreased until hatching time. The average frequency at 10 days was 280 bpm and 263 bpm at 19 days of incubation. Male embryos consistently had slower average heart rates than did females. No significant difference was observed in the average daily heart rate of embryos from 4 different genotypes, even though differences are apparent between adults of these genotypes. Mechanical disturbances or alterations in temperature had a marked effect on the heart rate of the embryo. Brief exposure to room temperature or slight injuries, such as those following insertion of electrodes, usually result in a decreased heart rate.

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