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SUMMARY

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A new ultrasensitive momentum transducer has been successfully adapted as a ballistocardiograph to measure the heart beat of avian embryos. Experiments at Ames Research Center have demonstrated that life can be detected as early as 4 days in the incubation period and monitored to maturity without damage to the avian embryo. Changes in heart-beat rate and intensity resulting from temperature changes or other external stimuli were readily detected by the instrument. The technique appears to open new avenues of investigation for application in such areas as vaccine production and drug research.

Author

INTRODUCTION

An ultrasensitive momentum transducer has been developed having unique characteristics which lend themselves to applications in many fields. Although several applications have been made at Ames, this report will be restricted to preliminary results of an application to biomedical research.

Biomedical researchers are currently using growing tissue, such as that found in chick embryos, to determine effects of vaccines and various drugs. One technique for determining the effect of drugs is to monitor the heart beat or muscular movement by use of probes inserted into appropriate areas for electromyographic measurements. This technique has numerous disadvantages, however. A second method, the visual or "candling" technique, appears to be the more generally accepted method in embryo drug research, but it too leaves much to be desired.

The investigation reported herein was undertaken to determine whether a piezoelectric transducer, developed at Ames as a micrometeoroid momentum detector, could be used to detect the heart beat of avian embryos. It was hoped that the sensitivity would allow the activity of the embryo to be monitored throughout the incubation period. Since the technique would not involve damage to the embryo, it would substantially improve the state of the art.

INSTRUMENTATION

Momentum Transducer

A photograph of the instrument used in the investigation is shown in figure 1, and a diagram of the sensing mechanism is presented in figure 2. The instrument operates as a spring mass system in which the primary mass is an avian egg cradled in a plastic basket attached to the top of a support stem. The stem is attached to a pair of piezoelectric beams which serve both as springs and acceleration sensors. This suspension provides essentially a single degree of freedom with sensitivity in only one direction.

The instrument was originally designed to provide the sensitivity needed to measure micrometeoroid impacts in outer space. The instrument can support a mass of 60 grams and still achieve a sensitivity of 10^{-5} dyne seconds (corresponding to 10 microvolts output) with a dynamic range of approximately 8 orders of magnitude (the momentum threshold corresponds to one thousandth of the impact achieved by a grain of table salt, weighing 150×10^{-6} gram, when dropped from a height of one centimeter).

The instrument was initially designed to measure randomly spaced impulses that recur at time intervals, which are large compared to the decay time constant of the spring mass system (approximately 1/20 second for the instrument tested). Since its application to chick embryo heartbeat measurements involves its use with periodic disturbances that recur at intervals of a few time constants, the peak output amplitude of the transducer is sensitive both to the magnitude of the heart beat and to its period. Further, since the momentum impulse cannot be assumed to be short compared to the period of the spring mass system, the output of the transducer used for these tests will be sensitive to the shape of heart beat. For these reasons, the tests described yield accurate data relative to existence of life activity and heart-beat rate but yield only qualitative information regarding the shape and magnitude of the heart beat.

Electronic Equipment

A block diagram of the instrumentation used in the experiments is shown in figure 3. The system utilized electronic equipment with an equivalent input noise level of 0.02 microvolt per cycle.

A dynamic force input (the impulse due to heart beat, for example) induces an exponentially decaying resonance of the spring mass system. The output is amplified, passed through a narrow band filter, and detected. A comparison of the detected and undetected forms of output will be discussed and illustrated subsequently in the results.

Calibration

Although the measurements were qualitative, the instrument was periodically calibrated as follows. A known dc voltage was applied to the lower beam of the transducer, inducing a beam displacement proportional to the applied potential. As the voltage was discharged, the suspended mass and the beams vibrated freely. The piezoelectric output from the upper beam provided a measure of the relative sensitivity of the instrument.

Filter tuning was also accomplished by use of the lower beam. The output from an oscillator on the lower beam oscillated the suspended mass and the beams at their resonant frequency; the filters were then adjusted for maximum output.

PROCEDURE

The instrument was essentially employed as a miniature ballistocardiograph. An egg was placed in a plastic cup attached to the stem of the instrument (fig. 1). Since the instrument is sensitive to acceleration due to internal mass movement, the accelerations resulting from action of the embryo heart were monitored by the instrument. The moving mass of the instrument was approximately 1/2 gram, whereas an average chicken egg is approximately 50 grams. Thus, differences in the masses of the eggs being tested appreciably altered the natural frequency of the instrument. It was necessary, therefore, either to retune the electrical filters in the system or to compensate by adding weights for eggs of lesser mass.

In order to use the instrument to the degree of sensitivity required for heart-beat measurements, spurious mechanical motions transmitted to the transducer by vibration of its supports or by acoustical coupling must be kept to a minimum. To isolate the transducer it was placed on a 1/4-inch-thick flexible foam urethane pad which rested on a heavy steel mass supported by 4 inches of urethane (as shown in fig. 4). This assembly was enclosed in a urethane foam box on the shelf of an incubator. The incubator was also supported on foam pads. To further reduce vibration and noise, the incubator was located within a double-walled soundproof room. The inner room was isolated from the outer protective enclosure by an air gap and supported by shock mounts from the floor. Most of the electronic equipment was located outside the room. Despite this isolation, if one gently tapped on the outside wall of the chamber, the instrument responded to the disturbance.

RESULTS AND DISCUSSION

Because the existing instrument was designed for a mass of 7 grams, Bobwhite quail eggs were used in the first experiments. These tests were necessarily crude in that ideal environmental conditions were not maintained and embryos of different incubation periods were not available. Further, the embryos were removed from an incubator, placed in a preheated box, and conveyed several hundred feet to the test room. Since the temperature of the test room was uncontrolled and approximately 25° F lower than optimum incubation temperature, the eggs gradually cooled with a resulting reduction of their vital activity. Despite this obviously crude procedure, the first results were quite encouraging. Results of these tests are shown in figure 5. Figure 5(a) is the direct output of the piezoelectric beam obtained from a 17-day embryo while figure 5(b) was obtained from a 15-day embryo. It can be noted that the 15-day embryo has a heart-beat rate approximately 75 percent faster than that of the 17-day embryo. The intensity of the recorded heart beat for the 15-day embryo is approximately 2-1/2 times that of the 17-day embryo. The difference in heart-beat rate and intensity was attributed to differences in temperature of the two specimens. The temperature of the ambient air was 95° F for the 15-day embryo and between room temperature (73° F) and 95° F for the 17-day embryo. The effects of temperature were observed as the egg environment was heated with a conventional hair dryer. The effects were even greater than those previously mentioned. Because the temperature effects were so pronounced, a standard incubator was employed thereafter (the test setup is discussed under Procedure).

The tests were terminated when the Bobwhite quail embryos became unavailable, but were resumed when an instrument capable of supporting a 60-gram mass became available. Thereafter, it was possible to use chicken embryos which were readily available at any desired incubation age. Further refinements to the instrumentation were also made. A detector was added to the system (see Instrumentation) to demodulate the output and provide a more conventional ballistocardiogram. A comparison of the direct and detected output wave forms is shown figure 6 for a 16-day chicken embryo.

The above results indicate that the first objective of the experiments was attained; namely, that the instrument is adaptable to such studies. The second objective was to establish how early in the incubation period life could be detected. It was found that in embryos of different incubation ages, life could be detected in approximately 4 days and a strong heart beat could be detected in 8 days. Figure 7(a) shows a typical 8-day heart beat. It may be noted that the magnitude is not quite so regular as in the more mature embryos. However, the response of the instrument to the activity of the 8-day embryo was well above the instrument noise level. This was ascertained by observing the output of the piezoelectric beam (fig. 7(b)) after substitution of an equivalent dummy mass.

The dynamic range of the instrument was adequate for use with both the 8-day embryo and the more mature embryos. For example, it can be noted from figure 7(a) (the 8-day embryo) and figure 8 (an 18-day embryo) that the 8-day embryo required the order of 80 times the sensitivity.

Observations were made periodically over a period of several hours for a pre-set orientation, and the heart-beat wave form remained essentially unchanged. It became obvious quite early in the investigation, however, that the embryos were definitely oriented in the egg shell and that the characteristic wave form changed when the egg was rotated with respect to its longitudinal axis. In order to observe the variation in heart-beat patterns with orientation, patterns were recorded with orientation changed (through 360° in increments of 45°) and are shown in figure 9. Although these records were obtained over a period of minutes and the angle not precisely set, the results tend to indicate the effects of embryo orientation. In all cases, however, when the egg was returned to its original orientation, the initial wave form was repeated. A further point of interest is that when an embryo is disturbed (e.g., by rotation), it responds to the stimulus by spontaneous muscular movement and changes in heart-beat intensity. It should be kept in mind also that the records reflect all forms of movement within the embryo. A sporadic muscular movement is quite pronounced in figure 9(c).

CONCLUDING REMARKS

Although the tests were quite limited and qualitative in nature, sufficient information has been obtained to ascertain the usefulness of the instrumentation for detecting motions of small living organisms. It has been demonstrated that without altering the embryo in any manner, the instrument can detect life within an embryo having a rigid enclosure. Activity of the embryo was detected 4 days after the beginning of incubation. The effects of temperature on the embryo were quite pronounced and could be readily observed. The effects of orientation were also readily observed. Because the technique does not harm the embryo, life could be observed from 4 days after incubation to the full development of the embryo.

While useful experiments can be performed with the single-component instrument developed for other purposes, a three-component instrument with improved transient response would have obvious advantages.

ACKNOWLEDGEMENTS

The author wishes to acknowledge gratefully the assistance given by Robert S. Jenkins in making the tests, and to Gordon Deboo for the electronic design of the Ames Instrumentation.

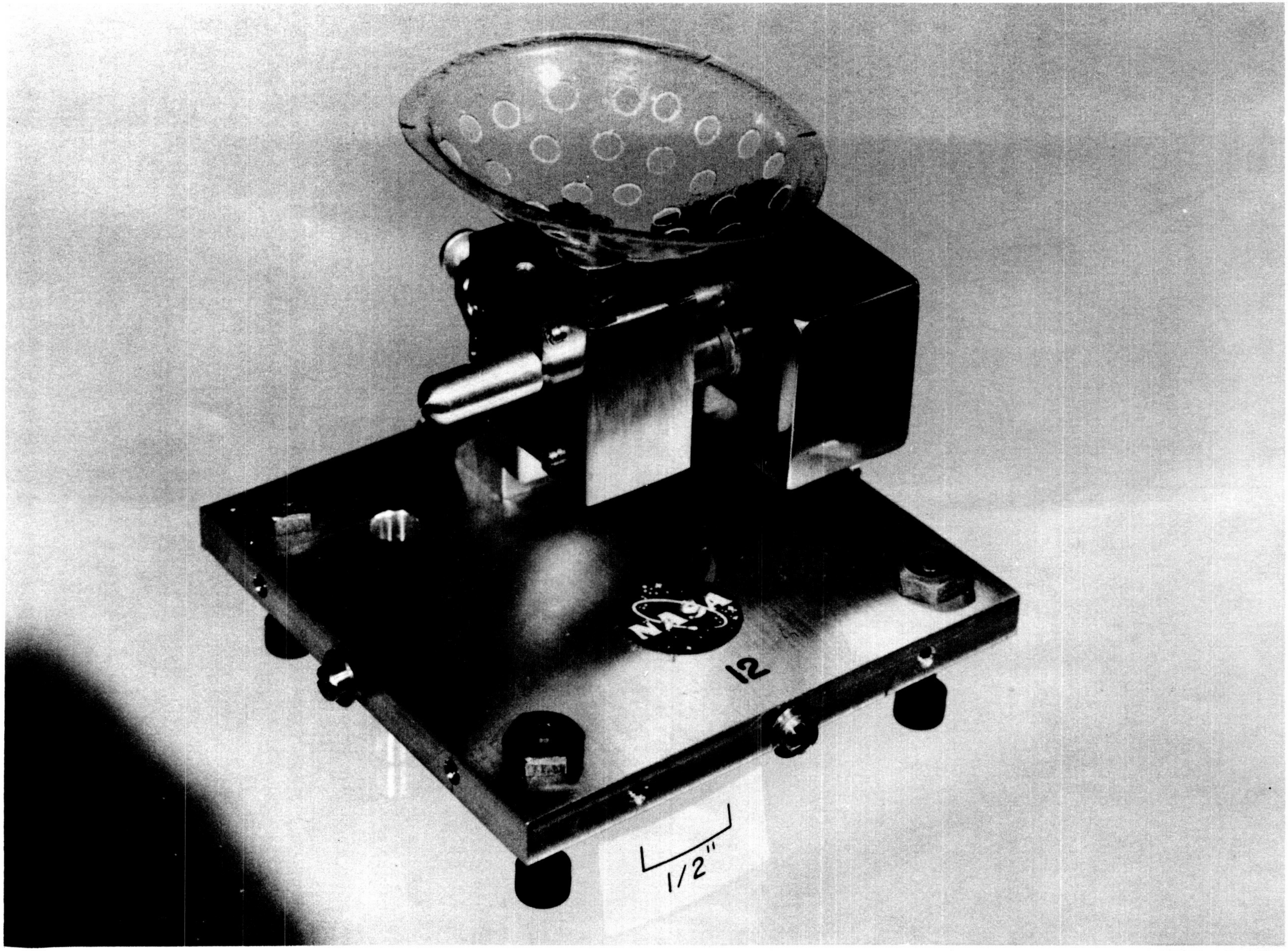

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Figure 1.- Momentum transducer adapted as an avian ballistocardiograph.

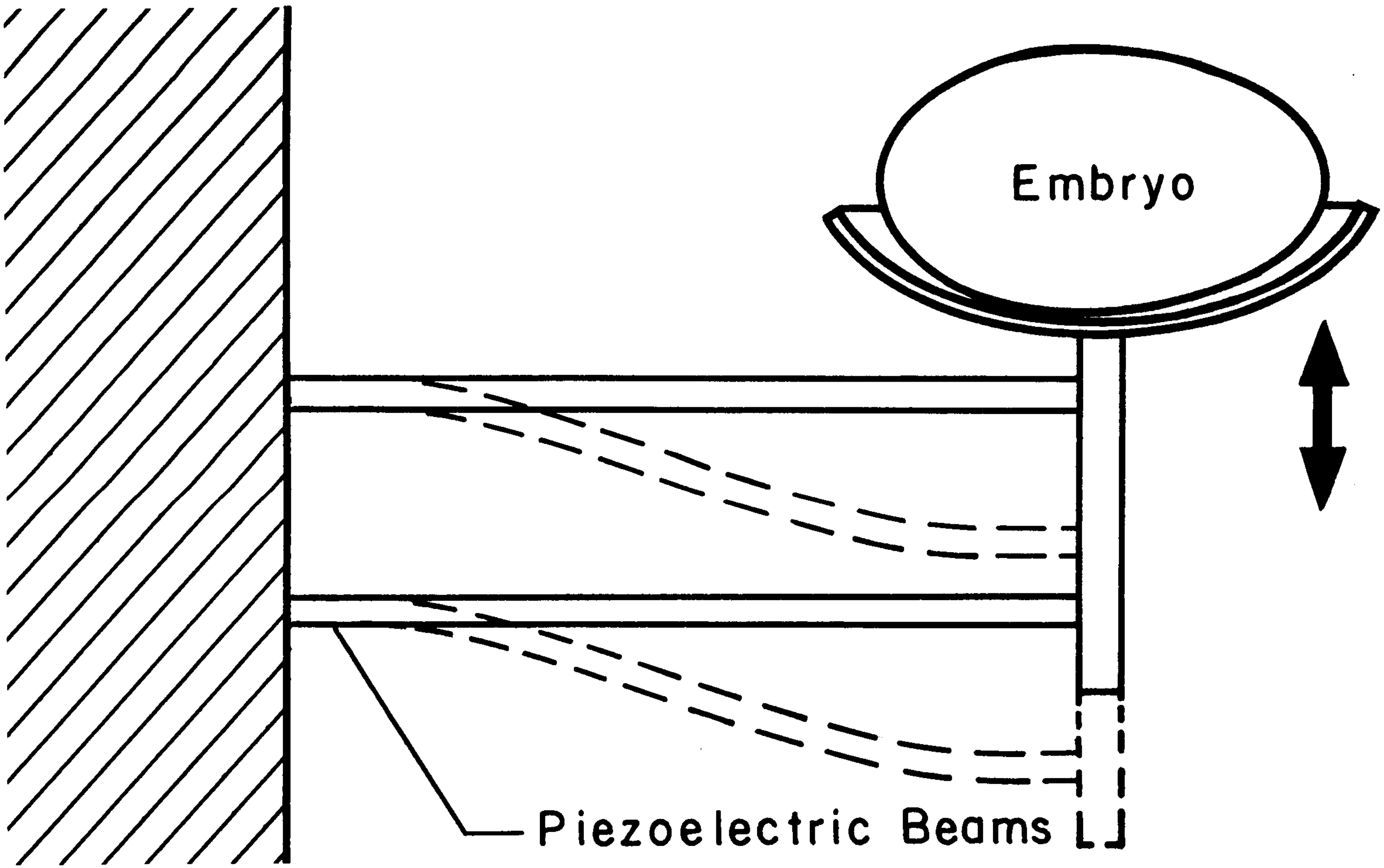

Figure 2.- Avian ballistocardiograph arrangement.

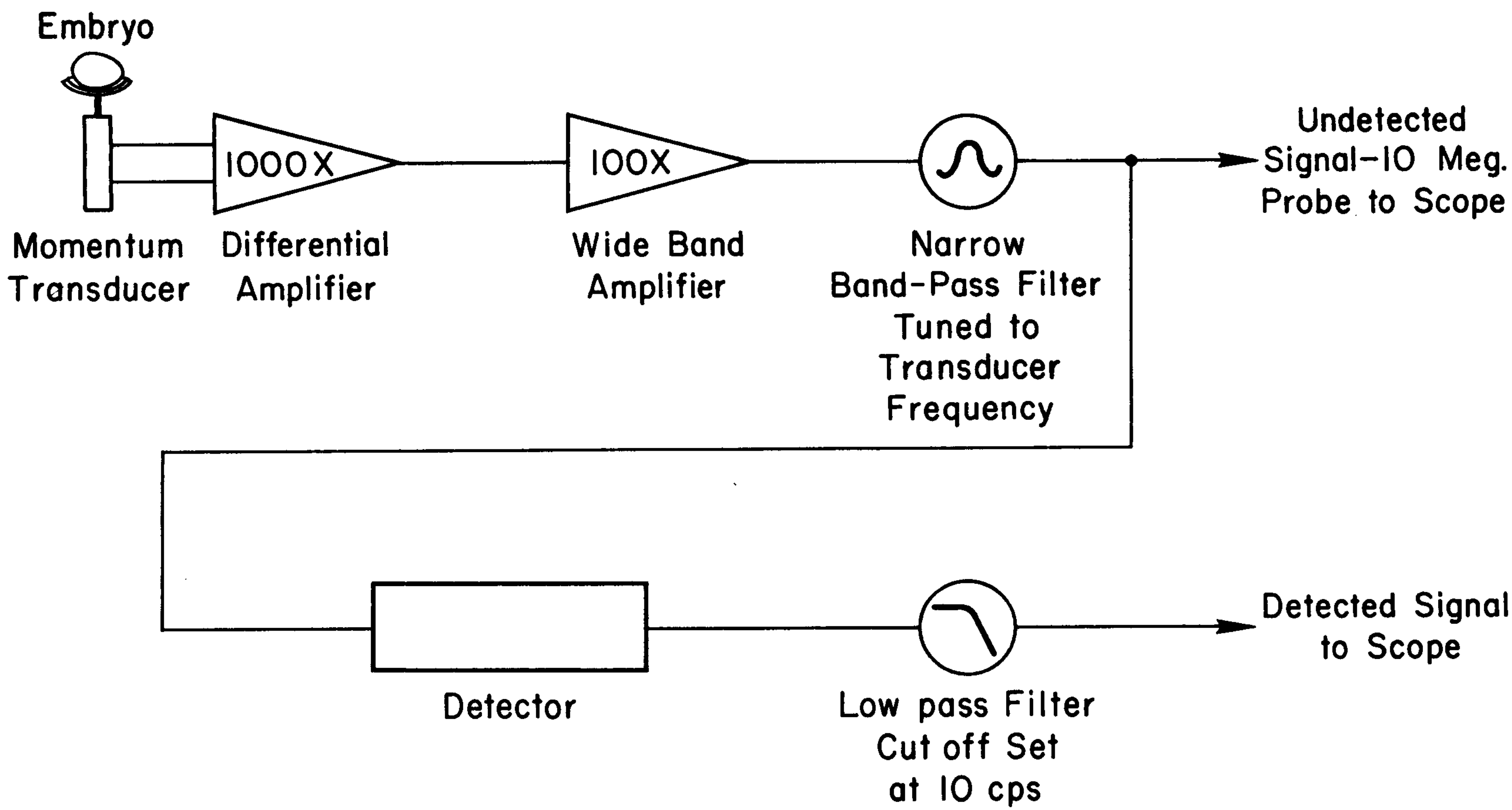

Figure 3.- Arrangement of electronic equipment.

Figure 4.- Incubator arrangement.

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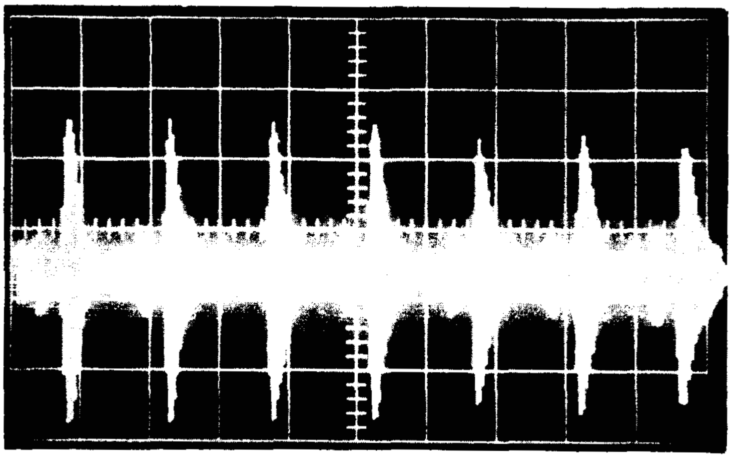
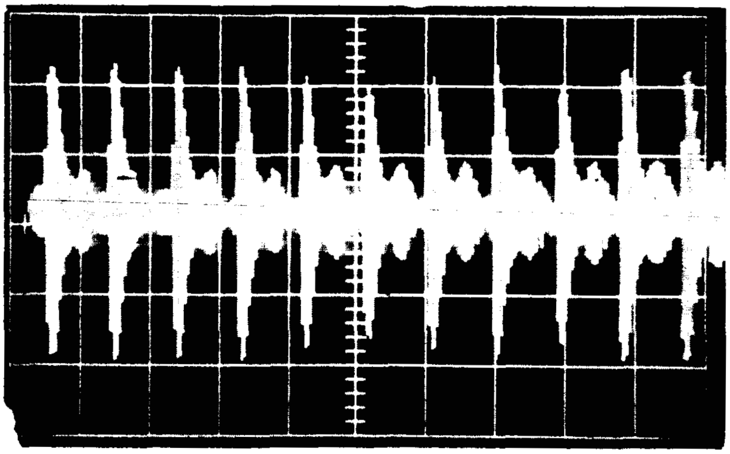


(a) 17-day Bobwhite quail embryo; horizontal scale = 0.2 sec/cm, vertical scale = 200 mv/cm.



(b) 15-day Bobwhite quail embryo; horizontal scale = 0.2 sec/cm, vertical scale = 500 mv/cm.

Figure 5.- Temperature effects on 17-day and 15-day embryos.

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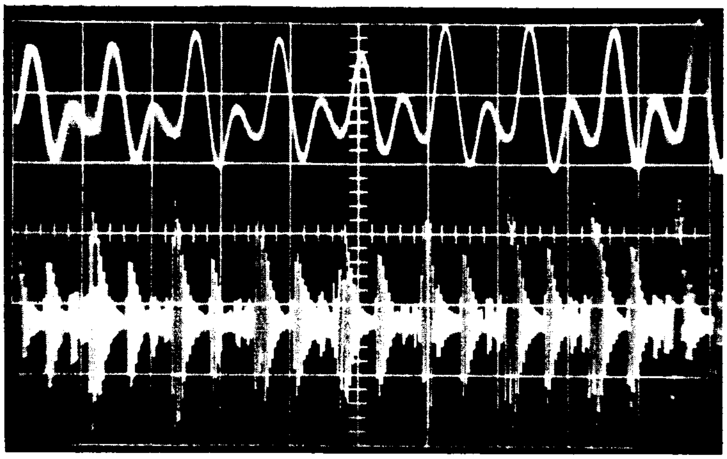
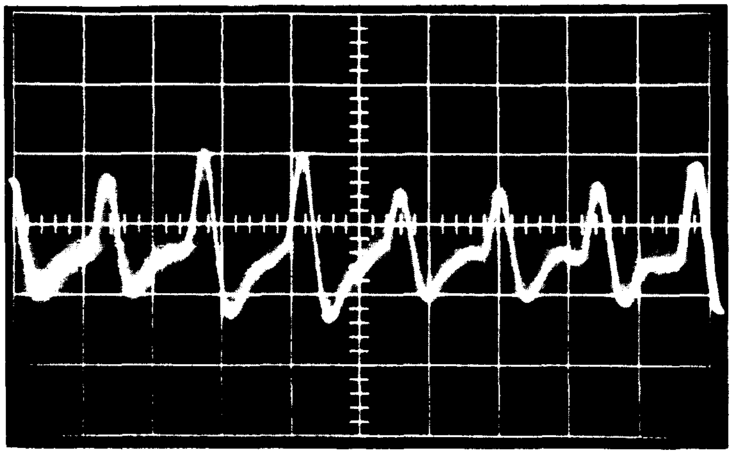
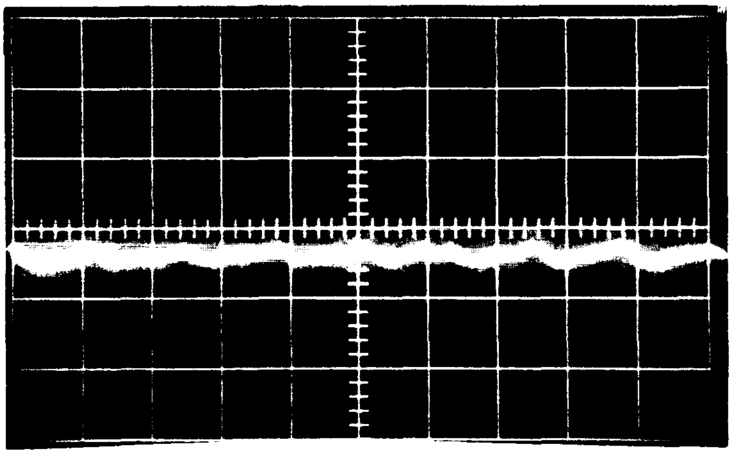


Figure 6.- 16-day chicken embryo; horizontal scale = 0.2 sec/cm, vertical scale = 50 mv/cm.



(a) 8-day chicken embryo, horizontal scale = 0.2 sec/cm,
vertical scale = 20 mv/cm.



(b) Noise level check with dead weight; horizontal scale = 0.2 sec/cm,
vertical scale = 20 mv/cm.

Figure 7.- Heart beat of young embryo and the associated instrument noise level.

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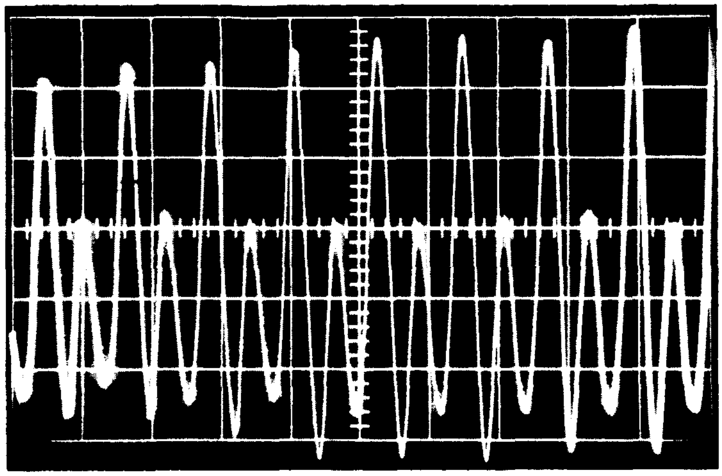

Figure 8.- 18-day chicken embryo; horizontal scale = 0.2 sec/cm, vertical scale = 500 mv/cm.

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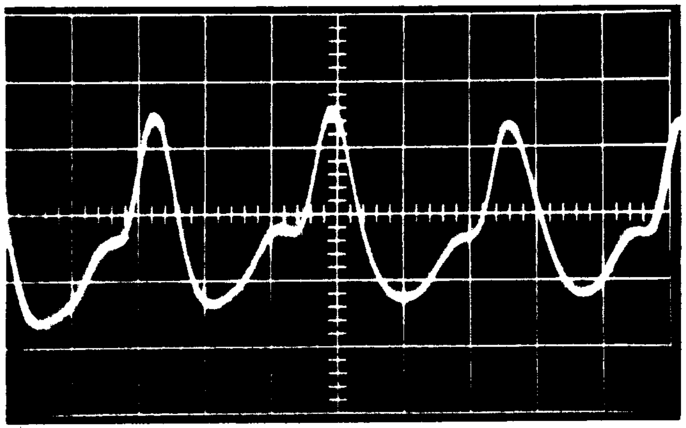
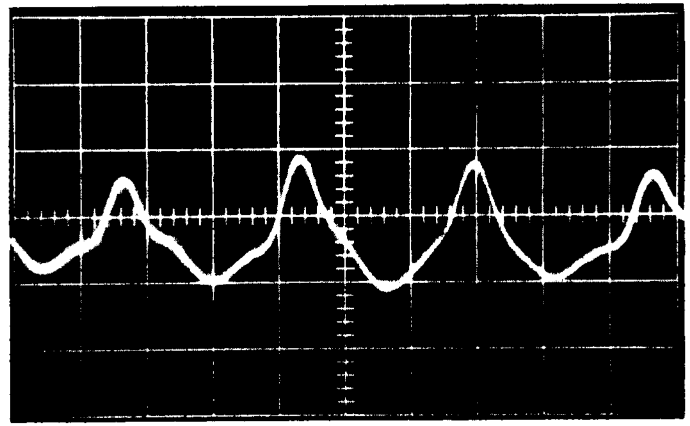
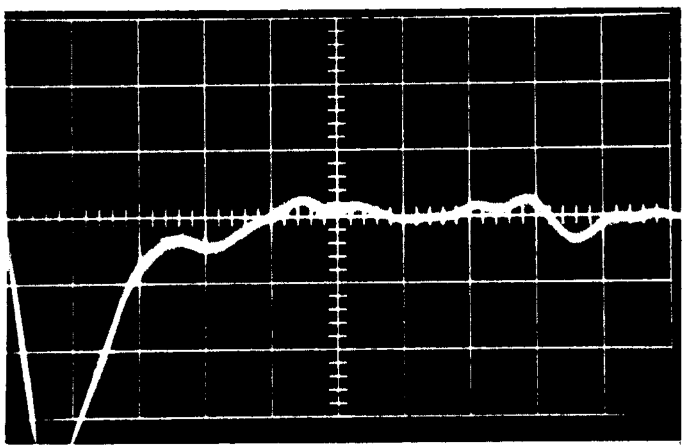


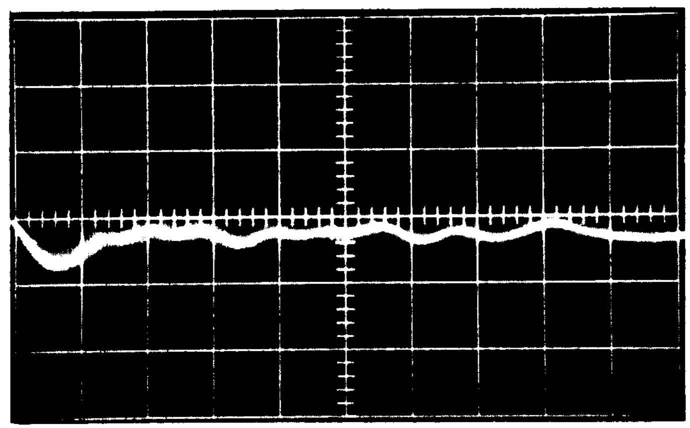
(a) Maximum signal, major axis horizontal



(b) Rotated 45°



(c) Rotated 90°



(d) Rotated 135°

Figure 9.- 15-day chicken embryo; horizontal scale = 0.1 sec/cm, vertical scale = 500 mv/cm.

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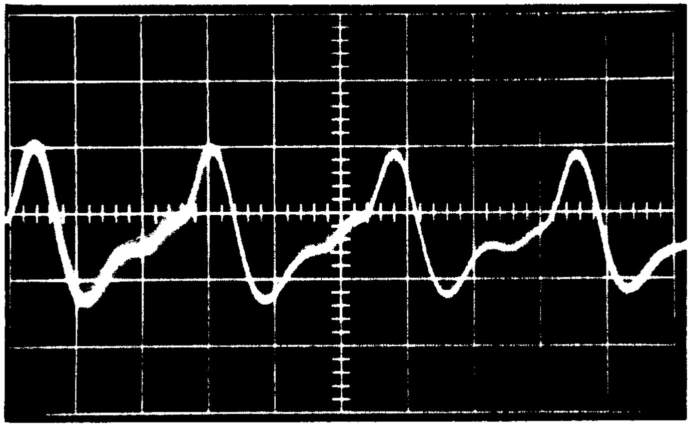
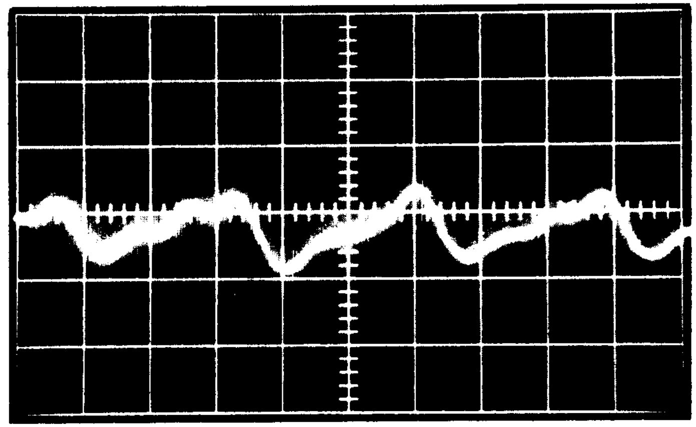
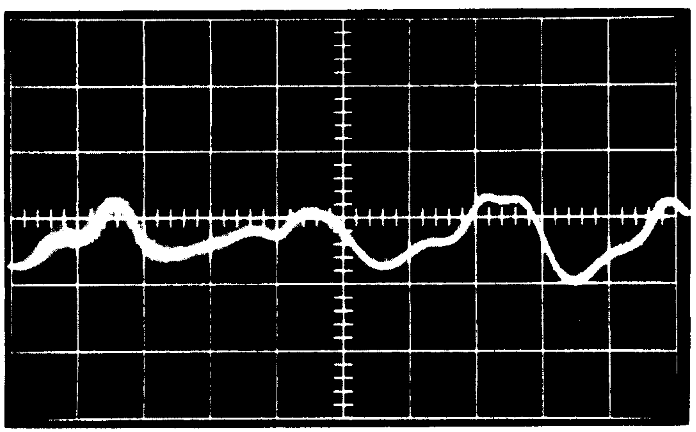


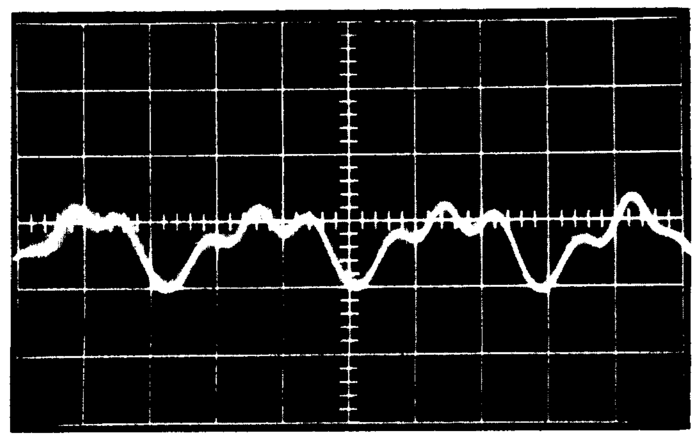
(e) Rotated 180°



(f) Rotated 225°



(g) Rotated 270°



(h) Rotated 315°

Figure 9.- Continued.

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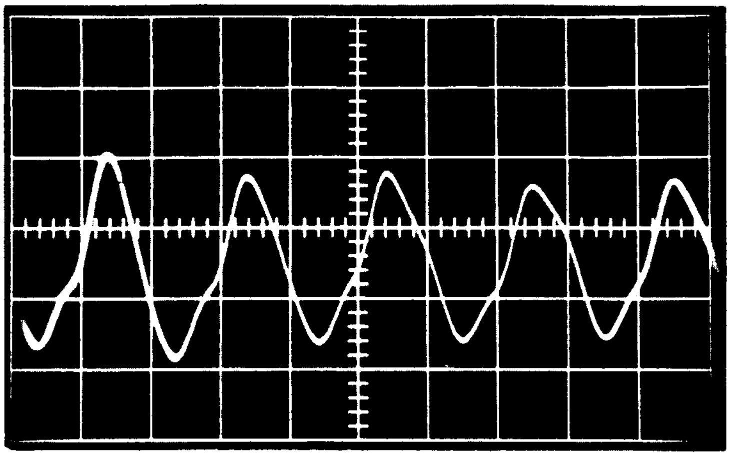
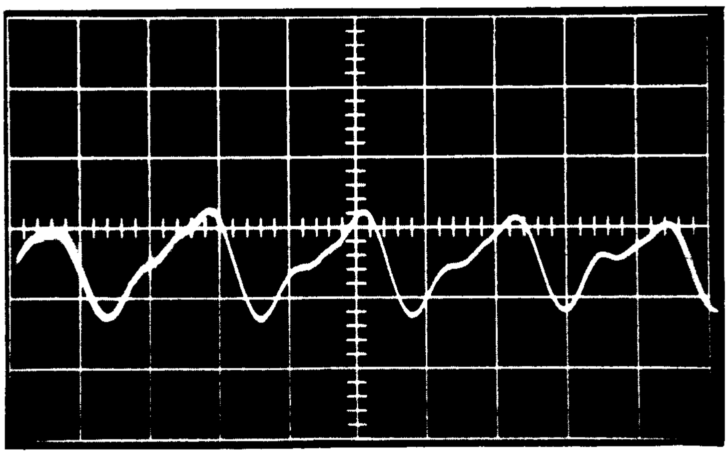


(i) Rotated 360°



(j) Placed on end

Figure 9.- Concluded.

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