cantly more erythropoietic (^{59}Fe from transferrin) than reticuloendothelial (^{51}Cr from erythrocytes treated with antibody) function in rat tibiae and fibulae and the opposite in rat vertebrae. This variation from our findings may reflect a difference in species or the fact that some reticuloendothelial cells phagocytose or sequester small particles differently from large particles; and Keene and Jandl did not compare these functions in the same animals.

If such similarities in distributions of erythropoietic and reticuloendothelial compartments also occur in animals with altered erythropoiesis, scintillation scanning of bone marrow (with ^{99m}Tc-sulfur colloid) will be an extremely useful tool both experimentally and clinically. Furthermore, combination of this technique with that of Suit (*6*) makes feasible an estimation of the total number of bone-marrow cells in each bone. If stem cells are similarly distributed, this information has important implications for experiments in radiation shielding and for radiation therapy.

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Amniotic Contraction and Embryonic Motility in the Chick Embryo

Abstract. *During part of the incubation period of chick embryos the amnion shows spontaneous contractions. Removal of the amnion on days 9, 10, and 11 has no effect on the amount of, or the cyclic aspects of, motility exhibited by the embryo. These observations question the importance of the amnion and yolk-sac as stimulative factors in the initiation and maintenance of cyclic embryonic motility at the ages studied.*

The amnion of the chick embryo is a nerve-free structure that contains smooth muscle fibers which are capable of spontaneous contraction (*1*). During the 5th day of incubation, the amnion becomes a complete sac within which the embryo and amniotic fluid are contained.

Whether the contractions of the amnion serve as a significant source of stimulation for "active" embryonic movements is a question that has not been answered by previous investigators. Both Preyer (*2*) and Windle and Orr (*3*) report that amniotic contractions and embryonic motility are independent of each other. However, both of these investigations were primarily concerned with problems other than the relation between embryonic motility and amniotic contraction. The first study designed specifically to investigate the relation of the amnion and yolk-sac to embryonic motility was that of Kuo (*4*).

Table 1. Means of percentage of embryo activity, duration of activity and inactivity phases, and percentage of amnion activity at each age for embryos with amnion intact and those with amnion removed.

Age of embryo (days)	No. of embryos	Amnion	Embryo activity (mean %)*	Mean duration (sec)		Amnion activity (mean %)†
				Activity phase	Inactivity phase	
9	10	Intact	46.4	24.0	27.8	94
		Removed	42.8	22.5	29.6	
10	10	Intact	59.3	30.2	21.5	89
		Removed	56.3	31.2	24.2	
11	10	Intact	72.3	44.3	16.5	82
		Removed	75.8	53.8	16.9	

* None of the differences are significant at the .05 level, with the use of the Wilcoxon matched-pairs signed-ranks test or the sign test (both two-tailed). † Means of the percentage of amnion activity derived from only the first recording of each embryo.

Kuo made recordings of the number of movements in 7-, 8-, and 9-day embryos with the amnion intact and with the amnion removed. He found that removal of the amnion reduced the frequency and amplitude of embryonic movements, and he concluded that the amnion (and yolk-sac) serve as significant sources of external stimulation for active embryonic motility. Since no statistical analysis was used and because sufficient details of the experimental procedure were not reported (*5*), the results are not unequivocal.

More recently Gottlieb and Kuo, in a study of development of behavior in duck embryos, reported that, "the action patterns of the embryo are also influenced by nonorganismic factors such as amnion contractions and movements of the yolk-sac" (*6*, p. 187). However, these authors were primarily concerned with specific body movements and patterns of movements, whereas my study is concerned only with the amount and periodicity of general somatic activity.

In earlier reports (*7*, *8*) it was stated that motility of chick embryos during certain stages of development is spontaneous (that is, nonreflexogenic) and cyclic. It was implied that this motility, which included amnion contractions, yolk-sac movements, and self-stimulation, was unaffected by exteroceptive stimulation of the embryo. For at least one stage of development (stage 37, day 11) (*7*), experimentally applied stimulation in no way altered the basic periodicity of motility of the embryo.

My investigation was undertaken in an attempt to replicate Kuo's study and to evaluate quantitatively the possible effects of amniotic contractions and yolk-sac movements on cyclic embryonic motility.

Thirty White Leghorn chick embryos were used. Ten embryos were observed at each of three stages of development: stages 35, 36, and 37 (*9*) which correspond to days 9, 10, and 11. Eggs were incubated in a large, forced-draft incubator at a temperature of 37° to 38°C and a relative humidity of approximately 70 percent. All observations were made in a plexiglass observation box with temperature and humidity held constant at values identical to those in the incubator.

An opening was made in the egg by cutting away a piece of shell over the air space. The inner shell membrane was removed and the embryo was exposed. The amnion was removed by carefully cutting a small opening in the chorioallantois with iridectomy scissors

and cutting out the major portion of the amnion. In most cases there was no hemorrhage. Amniotic contractions and yolk-sac movements were absent after this operation.

The embryo was allowed to remain undisturbed in the observation box for at least 30 minutes both after initial opening of the shell and shell membranes and after removal of the amnion. During these intervals the opening in the shell was sealed with parafilm. All operations were performed under sterile conditions.

Four 15-minute recordings were made on each of the 30 embryos, two before removal of the amnion and two after. Contractions of the amnion were recorded simultaneously with embryonic motility during the first recording of activity of each embryo. Embryonic motility and amniotic contractions were manually recorded on a Sanborn 4-channel polygraph. All observations were made through a binocular dissecting microscope. Only active (as opposed to passive swinging) somatic movements of the embryo were recorded. At the conclusion of the recordings each embryo was staged according to the Hamburger and Hamilton stage series.

Table 1 is a summary of the results. The mean percentage of activity refers to the total amount of time spent in activity by the embryo during a 15-minute recording period. Activity and inactivity phases have been defined previously (*8*); that is, if two activity phases are separated by 10 seconds or more they are treated as individual activity phases. If movements are separated by intervals from 1 to 9 seconds, they are considered as one activity phase.

At each age, the two values recorded with the amnion intact and the two with the amnion removed have been combined. This provides one mean value for recordings taken before removal of the amnion and one value for recordings taken after removal. With the Wilcoxon matched-pairs signed-ranks test and the sign test, no significant differences were found between the values for embryos with the amnion intact and those with the amnion removed.

Mean percentage of activity of the amnion was high at all stages that were studied. In fact, 22 out of the 30 embryos showed 100 percent amnion activity during the observation periods.

If contractions of the amnion and movements of the yolk-sac do, as Kuo suggests, stimulate active movements of the embryo, then the measure of the mean percentage of embryo activity should show a difference between the embryos with amnion intact and those with amnion removed. However, this is not the case. The discrepancy between these results and those of Kuo could be due to any of a number of factors: (i) the previously mentioned differences in methodology; (ii) the failure of Kuo to apply a statistical test of significance to his data; or (iii) the different ages of embryos used in the two studies. Kuo used 7-, 8-, and 9-day embryos whereas I used 9-, 10-, and 11-day embryos. However, the failure, in this investigation, to find any significant differences at the one age common to both studies (9-day) would seem to rule out the last possibility.

The cyclic aspects of motility (activity–inactivity phases) are also unaffected by removal of the amnion. The results are in agreement with experiments referred to above which showed that exteroceptive stimulation does not alter the periodicity of 11-day embryos. These results reduce the probability that sensory input (amnion contractions, yolk-sac movements, and exteroceptive stimuli), at least for the stages studied, is an important variable in cyclic, embryonic motility. The alternative hypothesis that cyclic motility is generated in the motor system is given further support.

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Chromosomes from Testicular Preparations of Lepidoptera

Abstract. *Typical elongate, beaded chromosomes have been observed in squash preparations of testicular tissue of the butterfly* Speyeria aphrodite *(Fabricius), the first demonstration of relatively uncondensed chromosomes in the Lepidoptera.*

No karyotypes with elongate chromosomes have so far been reported in Lepidoptera. To obtain chromosomes of a more "conventional" nature, as demonstrated in many other groups of animals, we have used the following technique.

The testis is removed from a living butterfly and is macerated thoroughly. The macerated tissue is then placed in a hypotonic saline solution, and the expanded tissue is transferred into a "soft" fixative that is composed of a mixture of methanol and glacial acetic acid (3 : 1, by volume). The fixed material is squashed between two

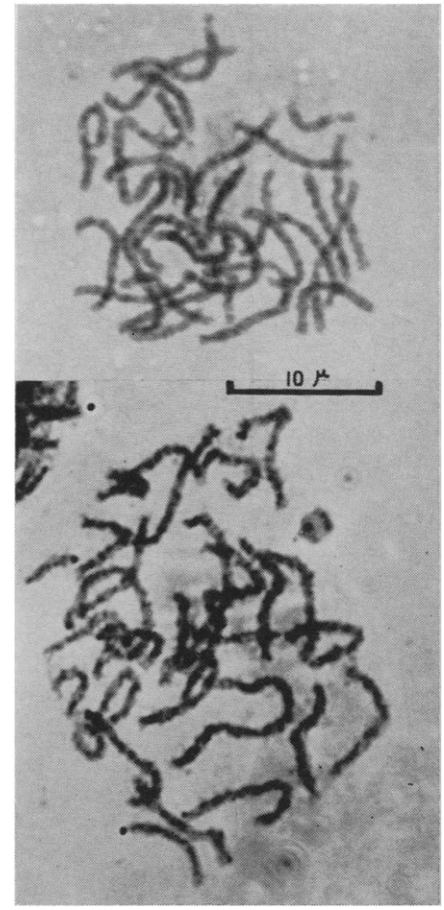

Fig. 1. Photomicrographs of two karyotypes from testicular squash preparations of *Speyeria aphrodite* (Fabricius) showing characteristic chromosomal morphology; the preparation was stained with Giemsa.