cells with lesser degrees of neoplastic change have more complex requirements, mesoinositol being prominent among the required components.

An evaluation of the significance of the present findings regarding the promotion of nodulation by mesoinositol must await further experimentation (7).

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Fibrillation and Potassium Influx

Abstract. Absolute influx and efflux of potassium-42 have been measured in isolated rabbit atria during acetylcholine-induced fibrillation. The efflux of potassium was increased three to four times; influx was not changed. The data are interpreted as indicating that an inhibition of active K uptake is not involved in the initiation of fibrillation, and that the process results from a marked increase in Na permeability.

Fibrillation has been induced by stimulating, at high frequency, isolated rabbit atria suspended in low potassium (K) media (1) in the presence of acetylcholine. Ion-exchange studies revealed that fibrillation began when the rate of net loss of K and gain of sodium (Na) exceeded critical values (2). Isotope investigations showed that with the onset of fibrillation the efflux of K reached a rate three to four times that of the spontaneously beating preparation (3). Net losses occurring under the conditions of the experiment prevented an accurate determination of influx. Therefore, we were unable to ascertain the nature of the permeability change involved in the process.

Recently a method has been devised (4) which permits an estimation of K^{42} influx with the onset of fibrillation. Absolute rates of influx were calculated by methods described by Keynes and Lewis (5). Influx is given by the product of the initial rate of entry of K^{42} to the tissue, the sensitivity of the counter, and the volume–to–surface-area ratio of the atrial fibers (6). The initial rate of entry can be obtained from the following relation:

$$\left(\frac{dy}{dt}\right)_{t=0} = \frac{Y}{T}\left(\frac{kt}{1-e^{-kt}}\right)$$

where Y is counts in the tissue after time T and k is the specific transfer coefficient obtained from efflux. During fibrillation, k was estimated to be of the order of 7.5 to 8.0×10^{-4} sec^{-1} (3).

Table 1 is a summary of our findings. First, it should be noted that acetylcholine increases both efflux and influx of K, whereas during fibrillation only an increase in efflux is obtained. Influx remains essentially unchanged. Thus, the changes induced by acetylcholine result from an increase in membrane permeability to K, while those that occur during fibrillation cannot be so interpreted. Earlier studies on the effects of temperature on efflux during fibrillation and acetylcholine treatment also suggested that different mechanisms were involved (7). A marked increase in Na permeability will explain the findings during fibrillation: Potassium leaves the tissue in exchange for sodium. This is in keeping with an earlier finding that the rate of entry of Na^{24} to atria was markedly increased (15 to 20 times) during the arrhythmia (7). These data suggested that the quantity of Na entering the tissue exceeded that of K which was lost. This would indicate that there was a sudden release of an anion in the tissue or, more probably, that membrane permeability to chloride is increased.

It should be noted that the mechanism proposed for the permeability change accompanying the onset of fibrillation is similar to that postulated for excitation and conduction in nerve (8) but differs in that K permeability is not increased. This is probably one of the factors responsible for the observed differences between the electrical properties of heart muscle and nerve (9).

Finally, it should be pointed out that the normal or slightly increased rate of influx during the early phases of fibrillation indicates that a depression of active transport is not a factor in the initiation of the arrhythmia, as was recently suggested by Goodford (10).

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Table 1. Effects of acetylcholine and fibrillation on the transmembrane flux of potassium. Fibrillation was induced by stimulating at 1200 count/min for 1 min. Atria were suspended in Ringer's solution containing 1.35 mmole of K^+ in the presence of acetylcholine (6.4×10^{-3} mole).

No. of observations	Experiment	Absolute flux (pmole cm^{-2} sec^{-1})	
		Influx	Efflux
8	Control	1.15 ± .08	4.32 ± .14
10	5 min after addition of acetylcholine (6.4×10^{-3} mole)	2.22 ± .13	7.88 ± .62
5	5 min after onset of fibrillation	1.20 ± .11	12.7 ± .48

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Failure of Nicotine to Affect Development of Offspring When Administered to Pregnant Rats

Abstract. Administration of nicotine to rats at any point in pregnancy has no apparent effect upon completion or duration of pregnancy, or upon body development, litter size, weight, or mortality of offspring. These results differ sharply from the effects in mice reported by others. The possible etiologic significance of anoxia in the malformations reported in mice is discussed.

Nishimura and Nakai recently reported (1) the development of a variety of skeletal anomalies, predominantly of the limbs, in the offspring (sacrificed at term, or examined at midpregnancy) of mice injected with a 0.1-percent aqueous solution of nicotine (0.025 mg/g) sometime between the 5th and 15th days of pregnancy. The percentage of congenital malformations, the number of pregnancies undergoing complete resorption, and the lethal effects of the drug upon the embryo were greatest when the drug was administered daily on days 9, 10, and 11 of pregnancy, although any or all of these effects could be produced, though to a considerably lesser

extent, by giving nicotine on any of the other days of pregnancy covered in the study. Litter size of mice whose pregnancies did not end in total resorption was found to be significantly different from that of the normal controls, though the average weight of such survivors, at birth, was relatively normal. Because the results in this laboratory differed markedly from those of Nishimura and Nakai just noted, I thought it appropriate at this time to report our experience with the use of nicotine as a potential teratogenic agent in the pregnant rat (*2*).

Rats from our stock colony (said to originate from Sherman strain and arbitrarily bred as a closed colony for at least 15 generations), raised on a standard Rockland Farms pellet ration, were mated between 4:30 and 5:00 each evening. The presence of sperm in the vagina (which was examined between 9:00 and 10:00 the following morning) was taken as presumptive evidence of pregnancy, and the morning of the positive smear was counted as the first day of pregnancy. Rats were injected subcutaneously, for 4 days, on the 9th, 10th, 11th, and 12th days of pregnancy, or daily for the first 20 days of pregnancy, with either 1.5 mg or 4.5 mg of nicotine per kilogram. A 0.1-percent aqueous solution of the chemically pure liquid alkaloid base (Fisher) was used for all dosages.

Preliminary findings indicated that a dose of 1.5 mg/kg would avoid the possibly complicating factor of convulsion in almost 100 percent of the injections but that this dose was nevertheless sufficiently effective to result in a gradually increasing flaccid paralysis (most apparent in the rear limbs), abolition of the placing reactions, and increase in rate of respiration. A dose of 4.5 mg/kg quickly resulted in loss of locomotor ability and in profoundly altered respiration. Mild clonic activity of the limbs, together with concave arching of the back and total-body spasms, was noted in approximately 30 percent of the injections. No deaths resulted from either dosage.

All animals were observed throughout pregnancy, and daily weights were taken in order to detect possible alterations in the course of pregnancy. Litters were carefully examined and weighed as soon after birth as possible. As controls for the treated animals, a small group of pregnant rats injected with saline (see Table 1) was run, and the offspring were examined similarly. Moreover, colony records of length of pregnancy, number of living and dead offspring in litter at birth, and weight at birth provided an essential background against which to evaluate these factors in the experimental litters.

Table 1 summarizes the effects of nicotine in the experimental and control groups. None of the nicotine treatments, regardless of dosage or of period of pregnancy covered, had an adverse effect upon litter size, weight of young at birth, or number of offspring dead at birth. In all cases the dead offspring appeared to be full-term animals, and their well-preserved condition suggested that death had occurred in the immediate perinatal period. In no instance was there a case of pregnancy undergoing complete resorption or, to judge by litter size at birth, any indication of a lethal effect of nicotine upon embryo or fetus. Duration of pregnancy was normal in every experimental animal.

Table 1. Effects of nicotine, administered subcutaneously to pregnant rats, on outcome of pregnancy and upon offspring examined at birth.

Treatment (dosage and days of pregnancy when injected)	No. of females treated	No. of females delivering*†	Av. length of gestation (day)‡	Total No. of offspring born	Total No. of offspring dead at birth†§	Distribution of dead by litter‖	Av. No. of offspring (live and dead) per litter‡	Av. wt. of living offspring at birth (g)‡§
1.5 mg/kg (9–12 days)	36	34(94.4)	22.8 ± 0.11	338	16(4.7)	3, 3, 4, 3, 2, 1	10.2 ± 0.35	5.3 ± 0.08
1.5 mg/kg (1–20 days)	12	11(91.7)	22.5 ± 0.02	116	1(0.9)	1	10.5 ± 0.56	5.2 ± 0.16
4.5 mg/kg (9–12 days)	7	5(71.4)	22.2 ± 0.20	52	1(1.9)	1	10.4 ± 0.59	5.2 ± 0.28
Saline controls#	7	5(71.4)	22.6 ± 0.25	56	0(0)	0	11.2 ± 0.58	5.3 ± 0.29
Colony record controls		56/68 (82.4)**	22.8 ± 0.18	545	28(5.1)	3, 1, 3, 3, 1, 7, 2, 2, 2, 1, 1, 1, 1	9.8 ± 0.43	5.5 ± 0.14 [††

* Rats which failed to deliver in these groups did not give any indication of pregnancy in terms of daily weight gain. Lack of pregnancy was confirmed by post-mortem examination of ovaries and uterus, 15 to 20 days after insemination.
† Percentage in parentheses.
‡ Standard error of the mean.
§ The term *at birth* refers to a period ranging from direct observation of offspring being delivered to as much as one-half day thereafter. In all cases weights were taken after the babies had an opportunity to suckle. The distribution of periods at which postnatal observations were first made did not differ significantly among the groups.
‖ Each entry represents the number of offspring dead in a single litter.
Volumes injected equivalent to those used in 4.5 mg/kg group.
** The denominator represents the last 68 females mated, of which 56 delivered litters. The failure of approximately one of every five inseminated females to become pregnant represents a reliable estimate of our long-term experience with the colony.
†† Based on 56 offspring from 6 litters.

In further contrast to the report of Nishimura and Nakai, not a single animal (of a total of 506 offspring) in any experimental series was found, on systematic gross examination at birth, to display any type of malformation. Post-mortem examination of animals dead at birth also failed to reveal evidence of congenital defect. It is not impossible that continued observation of the young after birth might reveal deficiencies in growth and development (*3*) or the presence of other defects—for example, decreased fertility (*4*). Detailed study of the offspring during growth, plus systematic post-mortem examination for the presence of malformations, is, however, still lacking.

The sharp difference between total absence of skeletal malformations at birth in our rats, as compared with the findings of Nishimura and Nakai in mice, may represent a striking species difference in response to the teratogenic potency of nicotine. Other than this, a possible basis for our results may be sought in the difference in dosage of drug used. The dose employed by the Japanese workers would amount to 25 mg/kg, or approximately six to 17 times the dose used by us. Doses based on those of Nishimura and Nakai with mice would, in our animals, uniformly produce severe convulsions, which, according to our experience, would be fatal in the majority of cases. In itself this suggests an interesting species difference in the maternal animal's tolerance for nicotine, though it does not clarify the basis for the difference in effects upon the fetus.

Nishimura and Nakai believe the malformations they find can be ascribed to the direct action of nicotine upon embryonic cells. In view of the known cardiovascular responses to nicotine, coupled with its curariform effect upon the muscles of respiration (as well as its depressive action on medullary respiratory centers), the possible role of anoxia in the production of these malformations also must be considered. Ingalls and his coworkers (*5*) have shown that exposure of the pregnant mouse to different degrees of frank anoxia, at various stages of gestation, results in a continuum of damage to the conceptus ranging from total resorption to anomalies present at birth. The latter involve not only striking defects of the nervous system but also clear abnormalities of the skeletal structure, including cleft palate and malformed vertebrae and ribs.

Whatever the intervening mechanism or mechanisms in the production of congenital malformations may be, it appears that large quantities of any of a wide variety of agents (*6*) administered to the maternal animal at particular stages of pregnancy, will frequently result in either the death of the conceptus or in defects present in the offspring at birth. The nature of these de-

fects in many instances does not appear to be specific for the *agent* used but may be more clearly related to the intensity of the treatment and the period of pregnancy at which it is given, as well as to the interplay of these factors with genetic determinants in the developing organism (*7*).

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Alginase in the Sea Urchin Strongylocentrotus purpuratus

Abstract. Viscosimetric evidence of alginase activity is given for the intestine and the intestinal contents of a sea urchin. The alginase activity of the gut wall and that of the contents of the gut differ in *p*H optima; this suggests that there may be two sources of alginase. The enzyme (or enzymes) depolymerizes algin.

Alginase may be a common digestive enzyme in invertebrates which feed upon brown algae. The presence of the enzyme has been noted in intestinal extracts of an abalone, *Haliotus giganteus*, and a sea urchin, *Sphaerechinus pulcherissima* (*1*), and in a sea hare, *Aplysia punctata* (*2*).

The alginase from Haliotus (*2*), like that of certain bacteria (*3*), appears to hydrolyze algin to free mannuronic acid, after a period of incubation. As with pectic-acid hydrolysis by pectin-polygalacturonase (*4*), the viscosity of the algin solution is much reduced before measurable reducing sugar appears (*2*). By analogy with the nomenclature of pectic enzymes (*4*), this alginase may be termed algin-polymannuronase.

In *Cryptochiton stelleri* (*5*) and in the sea urchin *Strongylocentrotus purpuratus*, alginase activity has not yet been demonstrated by the appearance of reducing sugar, in tests in which the Somogyi-Nelson reagents are used (*6*). However, we have found that intestinal extracts of the sea urchin very quickly reduce the viscosity of algin solutions.

The presence of an alginase in marine animals and production of this enzyme by marine decomposing microorganisms may have considerable ecological significance in the economy of the intertidal and subtidal zones. Waksman *et al.* claim that bacteria are most important in algin decomposition and assign a negligible role to marine fungi (*7*).

A further importance of alginase may lie in its use in clarifying the structure of algin, which is known to be a linear polymer of mannuronic acid, although details of the structure are still not fully known (*8*). Miwa (*2*) used the enzyme from *Haliotus* in a study of the anatomy of brown algae.

Because algin is a major constituent of brown algae, which, at times, make up a large portion of the diet of *S. purpuratus*, our purpose was to examine this animal for the existence of an alginase and to determine whether such an enzyme is active at the reported *p*H of the gut.

Crude enzyme preparations were obtained from (i) whole gut plus gut contents; (ii) the gut wall, washed five times with sterile sea water; (iii) the liquid gut contents; and (iv) the solid contents of the gut (in this case mostly coralline red algae). The filtered gut liquid contents were used directly. The other preparations were ground in a mortar with cold 0.5*M* tris buffer (tris-hydroxymethylaminomethane) at *p*H 7.5, then filtered through Whatman No. 1 and No. 42 filter papers before use.

These extracts were mixed with approximately 0.1 percent of sodium alginate (*9*), and subsequent changes in viscosity were followed by means of a rolling ball (Hoeppler type) viscosimeter. Decrease in viscosity was taken as evidence for digestion of the algin (*2*).

For the determination of activity with varying *p*H, tissues were ground in distilled water and filtered as before, then mixed with alginate made up in the appropriate buffer. Variation in the initial algin viscosity, especially marked in McIlvaine's buffers, was corrected for in the activity determinations. The digestion experiments never exceeded 4 hours' duration, and no preservative was used.

Potassium oxalate (final concentration 0.03 percent) was added to the gut liquid and gut solids extracts to eliminate the effects of calcium salts (from the coralline red algae) on the viscosity of the algin solutions. The same amount of oxalate, added to extracts low in calcium, was without effect on the enzyme activity.

Tests for reducing-sugar production during the reaction period were made by the Somogyi-Nelson method (*6*). Tests for mannuronic acid were made on aliquots, after precipitation of polyuronides with 10 percent calcium chloride, in a naphthoresorcinol test (*10*).

All of the extracts appeared to contain an enzyme or enzymes capable of digesting algin. At the same time, no increase in reducing sugar could be demonstrated, nor did any calcium-soluble, naphthoresorcinol-positive products appear. Only the marked decreases in viscosity observed indicated that digestion had occurred. Again, by analogy with pectic enzyme nomenclature, this alginase may be called an algin depolymerase.

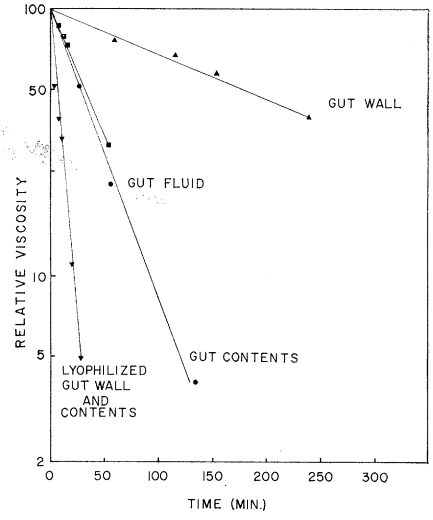

Fig. 1. Reduction in viscosity of sodium alginate solution in the presence of alginase extracts from the sea urchin *Strongylocentrotus purpuratus*. Relative viscosity = (100 minus percentage of viscosity change). Reaction mixtures contained: (i) gut fluid, gut solids, and gut wall; (ii) 3 ml of extract, 14 ml of 0.1-percent sodium alginate, and lyophilized gut wall and contents; (iii) 10 ml of extract, 20 ml of sodium alginate.

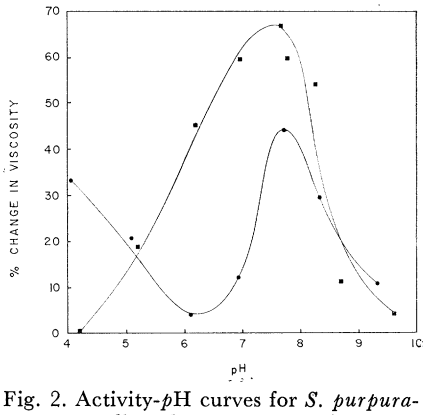

Fig. 2. Activity-*p*H curves for *S. purpuratus* gut wall and gut contents (squares) and gut wall (circles). Tris buffer was used for *p*H 7.5 and above; McIlvaine's buffer, for *p*H 7.5 and below. Reaction mixtures contained: (i) gut wall and gut contents; (ii) 4 ml of enzyme preparation, 5 ml of buffer, 10 ml of 0.1-percent sodium alginate, gut wall; (iii) 1.5 ml of enzyme preparation, 5 ml of buffer, 11 ml of sodium alginate. Reaction period, 30 min.