trols did, but much less than was incorporated by a Hurler cell strain. When exposed to an experimental medium for 6 days prior to labeling with [^{35}S]sulfate at 3, 6, and 24 hours, GALT cells incorporated twice as much of the isotope as did control cells when glucose was present (by itself or mixed with galactose), and only half as much when exposed to a galactose medium. In contrast, control cells incorporated slightly more [^{35}S]-sulfate when galactose was present (see Fig. 1a).

Exposure to media containing glucose, galactose, or both for 4 to 6 days and then to [^{35}S]sulfate for 24 hours has little effect on control cells, whereas GALT cells consistently incorporate much less isotope when exposed to a galactose medium (see Fig. 1b).

Decreased sulfate incorporation by GALT cells on a galactose medium is not an effect of carbohydrate starvation. Control and GALT cultures exposed to a hexose-free medium for 4 to 6 days and to [^{35}S]sulfate for 24 hours incorporated amounts of isotope into the acid-insoluble fraction that were similar to those incorporated by replicate cultures on a glucose medium (see Fig. 1). Thus it is more probable that the decreased sulfate incorporation by GALT cells that results from exposure to galactose is a consequence of galactose toxicity.

The incorporation of [^{3}H]uridine into the acid-insoluble fraction did not distinguish control and GALT cells, although on a galactose medium, both kinds of cell slightly decreased their incorporation of [^{3}H]uridine (see Fig. 2, triangles). Thus the two different isotopes are incorporated differently in the presence of galactose.

The isotope incorporation results are presented in Fig. 2. These data were obtained by calculating the ratio of isotope incorporation by cells on the galactose medium to that incorporated by the same cells on the glucose medium and expressing this ratio as a percentage. The Hurler cell strain incorporated excessive amounts of [^{35}S]sulfate on both the glucose and galactose media. The GALK cell cultures behaved like control cells, demonstrating that the differences observed for the GALT mutants are not obvious when galactose cannot undergo phosphorylation. The solid circles in Fig. 2 represent data obtained with the ethanol precipitation technique (routinely used to detect excess sulfate uptake by cultured cells deficient in hydrolytic enzymes, which degrade glycosaminoglycans). Although these values are higher than those obtained with the TCA precipitation method (Fig. 2, open circles), the difference between control and GALT cells is similar.

These data demonstrate that the absence of galactose-1-uridyltransferase activity or the accumulation of intracellular galactose-1-phosphate can produce secondary metabolic alterations in sulfate metabolism. Furthermore, the decreased incorporation of sulfate into acid-insoluble macromolecules occurs at a time when neither gross cellular morphology nor another biochemical pathway ([^{3}H]uridine incorporation) appears to be affected. Although the alterations reflected by reduced [^{35}S]sulfate incorporation remain to be defined in molecular terms, such changes could be related to the toxic mechanisms responsible for the tissue pathology associated with classical galactosemia.

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Meiotic Maturation in *Xenopus laevis* Oocytes Initiated by Insulin

Abstract. *Insulin can induce meiotic division in* Xenopus laevis *oocytes. This effect shows the specificity expected of a receptor-mediated mechanism. It is potentiated by ethynylestradiol, a steroid antagonist of progesterone (the natural hormone that provokes meiosis). The* Xenopus laevis *oocytes may serve as a model for the study of the poorly understood effect of insulin on cell division.*

Progesterone and other steroids can induce meiotic maturation of amphibian oocytes in vitro. They act at surface membrane sites to trigger meiosis (*1*), possibly by calcium movements or translocation from membrane stores, or both (*2-4*). Either cholera toxin, which increases adenosine 3′,5′-monophosphate (cyclic AMP) in oocytes, or cyclic AMP derivatives injected into *Xenopus laevis* oocytes hinders progesterone-induced meiosis in vitro (*5*) by interfering with the formation or activity of the maturation promoting factor (MPF). This factor develops after exposure of oocytes to progesterone and, in turn, initiates germinal vesicle breakdown (GVBD) and related phenomena of oocyte maturation (*1, 4, 6*). It is now reported that insulin can mimic the effect of progesterone on oocyte maturation.

Full-grown oocytes free of follicle cells were obtained from ovaries treated with collagenase (*6*), and were incubated at room temperature in Barth medium containing insulin (monocomponent, pork insulin, Novo). Groups of 50 oocytes were scored for GVBD after various periods of incubation.

Studies with 35 nM to 7 μM insulin (5 mU/ml to 1 U/ml) showed a dose-effect relationship with an apparent ED_{50} (50 percent effective dose) of 2 μM (Fig. 1A). The efficacy of the highest concentrations of insulin (up to 70 μM) remained less pronounced than that of progesterone used at lower concentrations, even when the oocytes were obtained under various conditions of collagenase treatment, or when the ovaries were dissected manually (data not shown). It is possible that the polysaccharide vitelline membrane impaired access of insulin to the oocyte surface or that insulin was progressively degraded during incubation. We therefore added fractions of insulin at different times of incubation, such as at 0, 2, 4, and 6 hours, and found the percentage of GVBD to be ~ 50 percent larger than when the total dose of hormone was introduced at time 0. This suggested a progressive time-dependent destruction of the hormone. The efficacy of insulin still remained lower than that

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Table 1. Potentiation of insulin-induced maturation in *Xenopus laevis* oocytes by 17α-ethynylestradiol. Results are expressed as percent GVBD.

Hours of incubation	Insulin			EE		Insulin (3.5 μM) + EE (10 μM)	Insulin (3.5 μM) + EE (5 μM)	Insulin (1.4 μM) + EE (10 μM)	Insulin (1.4 μM) + EE (5 μM)	Insulin (0.7 μM) + EE (10 μM)	Progesterone (50 nM)
	3.5 μM	1.4 μM	0.7 μM	10 μM	5 μM						
8	0	0	0	2	0	6	0	7	0	7	2
10	0	0	0	2	0	12	0	12	0	7	10
16	0	0	0	2	0	44	5	37	0	10	28
33	23	15	9	2	0	97	59	92	45	80	94

of progesterone, even for longer periods of incubation (30 to 35 hours), but it is known that oocytes become altered after such long periods. We also observed that maturation proceeded faster with progesterone than with insulin (Fig. 1B), suggesting that the two hormones trigger meiotic division by two different mechanisms.

We then conducted experiments to verify that the maturation in insulin-exposed oocytes was truly comparable to that of progesterone-exposed oocytes. First, a similar typical profile of labeled proteins was found after electrophoresis (*7*). Second, the introduction of 50 nl of the cytoplasm of insulin-treated oocytes into recipient oocytes induced 100 percent GVBD after 2 to 3 hours (*5*), showing that MPF was formed. Third, as observed for progesterone (*1*), no maturation occurred when insulin was injected into the oocyte (final intracellular concentration up to 70 μ*M*) or when cycloheximide (3 μg/ml) was added to incubated 7 μ*M* insulin. Fourth, cholera toxin (50 p*M*) was also able to antagonize the maturation induced by insulin (up to 7 μ*M*) that was added 30 minutes after the toxin (data not shown). Finally, cytology of insulin-exposed oocytes revealed in most cases the existence of a single and normal spindle moving to the cortex (not shown); it is noteworthy, however, that a double spindle was sometimes observed, as in oocytes exposed to nonsteroidal inducing agents (*8*).

We next investigated the specificity of the effect of insulin on oocyte maturation, because in most target cells (*9*) the apparent affinity of the hormone is ~ 10^3 more elevated than that observed in the present system. However, the micromolar range is itself 10^{-3} the size of the millimolar range at which nonsteroidal drugs are usually active. Denatured insulin did not provoke meiotic maturation. Denaturation was achieved by treating the hormone with 10 m*M* mercaptoethanol overnight at room temperature (*10*). After dialysis, a radioimmunoassay showed > 99 percent denaturation (*11*). Two modified insulins (*12*), which display 1 to 2 percent of the affinity of insulin for usual receptors (*13*) showed no maturational activity even at concentrations up to 7 μ*M*. Negative effects were also obtained when oocytes were exposed to epidermal growth factor (EGF) and fibroblast growth factor (FGF), each used at concentrations of 0.1, 1, and 10 μg/ml in buffer containing bovine serum albumin (5 mg/ml). In contrast to the above data, chick insulin applied at concentrations of 3.5 and 7 μ*M* displayed the same effect as pork insulin. Finally, proinsulin had a constant lower activity than insulin upon oocyte maturation (Fig. 1C), a result comparable to those obtained with proinsulin in other systems (*14*). Thus many data suggest a correlation between the effects of insulin derivatives on mammalian metabolic systems and on *Xenopus laevis* oocyte maturation. It is therefore possible that the insulin action for triggering amphibian meiosis is mediated by a receptor mechanism, although such a receptor has not yet been described in amphibian oocyte membrane. Recent studies with high-resolution chromatography have indicated that most insulin preparations available are not homogeneous (*15*).

We also studied the effects of a synthetic estrogen, 17α-ethynylestradiol (EE), applied simultaneously with in-

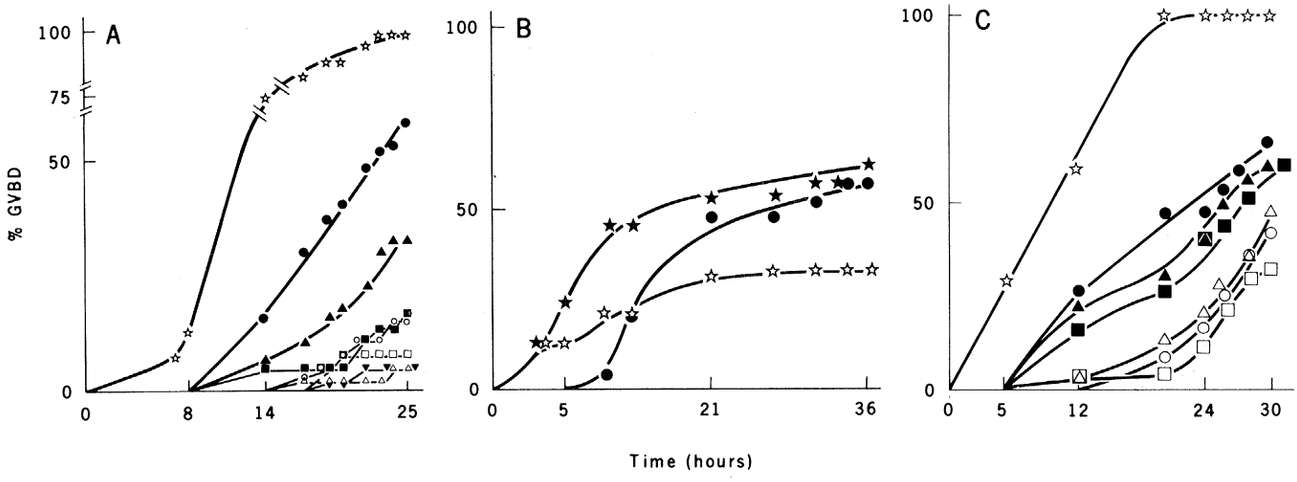

Fig. 1. Effect of hormones on *Xenopus laevis* oocyte maturation. In all cases pools of 50 oocytes from the same female were exposed to various doses of hormone. The percent GVBD was scored after different incubation periods. (A) Effect of various doses of insulin. Insulin concentrations: ●, 7 μ*M*; ▲, 3.5 μ*M*; ■, 1.4 μ*M*; ○, 0.35 μ*M*; □, 0.14 μ*M*; △, 70 n*M*; ▼, 35 n*M*. ☆, Progesterone control, 1 μ*M*. (B) Comparison between progesterone and insulin action. The curves show that 50 n*M* progesterone (★) and 3.5 μ*M* insulin (●) give the same plateau value, thus allowing a straight comparison of the first part of the response curves. Progesterone-induced GVBD occurs earlier than insulin-induced GVBD. This result is observed even when the plateau value obtained with 10 n*M* progesterone (☆) is inferior to the one reached with insulin. (C) Effect of proinsulin. Insulin concentrations: ●, 7 μ*M*; ▲, 3.5 μ*M*; ■, 1.4 μ*M*. Proinsulin concentrations: ○, 7 μ*M*; △, 3.5 μ*M*; □, 1.4 μ*M*. ☆, Progesterone control, 1 μ*M*.

sulin to *Xenopus laevis* oocytes. We found previously that EE was a very weak agonist compared to progesterone, and an antagonist of progesterone when the two steroids were used together, implying a competition for possible "steroid sites" on the oocyte membrane (*1*). When insulin was substituted for progesterone, no antagonistic effect of EE was observed (Table 1). Rather, we observed that EE potentiated the effects of insulin in a dose-dependent manner. The efficacy of insulin was comparable to that of progesterone and the lag before the first GVBD was similar to that observed with progesterone. If EE interacts with the steroid sites (*1*) and insulin interacts with a typical insulin receptor, and both are in the oocyte membrane, then there might be some interaction between these two loci or some cooperation between effects initiated at two separate places.

It has been suggested that in "metabolic" target cells, calcium may play the role of second messenger in insulin action (*16*) and that cyclic AMP concentrations decrease in response to insulin (*17*). These two possibilities are interesting to consider, because Ca^{2+} (*2–4*) and cyclic AMP (*5*) have both been found to interfere with the effect of progesterone on meiosis. The amphibian oocyte may thus serve (unexpectedly) as a model system for the study of the poorly understood mechanism of insulin action.

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Mutual Repulsion Between Moving Visual Targets

Abstract. *When two spatially intermingled sets of random dots move in different directions, the direction of each set may be misperceived. Observers report that each set of dots appears to move in a direction displaced by as much as 20° from the direction of its companion set. Probably the result of inhibitory interactions, this mutual repulsion occurs at a central site in the visual system and may normally enhance discrimination of direction.*

Inhibition is a nearly universal component of sensory systems (*1*), permitting coarsely tuned neural elements to support discriminations more acute than would otherwise be possible (*2*). A by-product of inhibition's role in discrimination is a large set of perceptual distortions in which differences between two simultaneous stimuli are exaggerated perceptually. Such distortions include Mach bands (*3*) and illusory expansions of acute angles (*4*). Here we report an analogous newly discovered and powerful distortion in the domain of motion: an exaggeration of the angular difference between visual targets that move in different directions relative to one another.

Our targets consisted of two sets of random dots presented under computer control on a cathode-ray tube (CRT). Each set contained 200 bright dots that moved continuously as a unit in a characteristic direction. Within a set, all dots maintained a fixed spatial arrangement as they moved along parallel paths (*5*). In preliminary research, observers described the direction of each set of dots with reference to an imaginary clock face. When the two sets of dots moved in different but similar directions, the angular differences in direction were markedly exaggerated; this misperception, which was reliably reported by all six observers, involved an apparent expansion, or broadening, of the angle in a manner that suggests some form of mutual repulsion from the actual directions of motion. With certain angular differences, the misperception exceeded 20° for each set of dots. We then set about to make systematic quantitative measurements of the mutual repulsion, simplifying the task so that observers would be required to report the direction of only one of the two moving patterns.

Dots were presented behind an aperture of 9.2° diameter for 1 second. Seen against a uniform veiling background of 3 cd/m^2, the dots had a contrast approximately 50 times their own threshold. One set of dots always moved horizontally from left to right across the CRT (the ends of the CRT were functionally connected in a wrap-around fashion); the second set moved in a direction θ, for which increasing θ indicates directions more counterclockwise relative to the rightward-moving dots. Regardless of direction, all dots moved at 4° per second.

In the first experiment, observers viewed the CRT binocularly, holding their gaze steady on a fixation point in its center. After a 1-second exposure, the dots were extinguished, and the observer used a protractor scale ringing the CRT to estimate the direction of just the dots that had moved in direction θ. The actual value of θ varied randomly from trial to trial. The same three observers served in all experiments. Two were paid volunteers, naïve as to the purpose of the research; the third was W.M. Our main findings were verified by the informal reports of several other observers as well.

Almost always, dots that actually moved in direction θ were judged to move in a direction more counterclockwise than θ. This error in perceived direction increased rapidly with θ, peaked in the vicinity of 22.5° and gradually declined thereafter [F (1, 2) = 33.87, $P < .05$] (Fig. 1). The maximum misperception of direction, about 20°, is nearly an order of magnitude greater

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