periments have demonstrated that 3-OH-kynurenine is transferred into the fat body from hemolymph.

In young pharate pupal stage A2 the hemolymph concentration of 3-OH-kynurenine is about 220 nm/ml. This concentration would lead to chromatid breakages[4] and a decrease in thymidine incorporation[5] in human cell lines. Thus at least the biological significance of accumulating 3-OH-kynurenine in the fat body might be a protection of the developing adult against an excess of this compound. Possibly the same is true for kynurenine. In agreement with a role as a temporary storage site for precursor material needed later on, the fat body histolyses most intensively during the pupal phase just prior to the main onset of ommochrome synthesis in the developing compound eyes[2,6]. The total amount of tryptophan metabolites found in the fat body of the late pharate pupal stage would be sufficient to synthesize about 60% of the ommochromes found in the heads of adults 2 days after eclosion[9]. There are first indications that the accumulation is under direct or indirect control[18].

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Vasopressin does not stimulate fatty acid synthesis in mouse mammary explants[1]

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Summary. Fatty acid synthesis in mammary explants from pregnant mice increased after incubation with insulin, cortisol, and prolactin. Replacement of prolactin with vasopressin did not stimulate this activity. Prolactin treated with an anti-vasopressin antiserum had activity similar to untreated prolactin. Thus, stimulation of this lactogenic function by prolactin is not the result of its contamination with vasopressin.

Prolactin regulates a large number of functions in different tissues[2]. One well-studied function of this hormone is its stimulation of epithelial cell differentiation in mammary tissue. Treatment with prolactin either in vivo or in vitro can increase the synthesis of several biochemical markers of mammary differentiation[3]. In mouse mammary tissue, one marker of differentiation is the synthesis of medium chain fatty acids (MCFA)[4]. These fatty acids are not produced by mammary epithelial cells in virgin animals. In lactating animals, however, mammary epithelial cells synthesize a high proportion of MCFA, which are unique to this tissue and its secretions (milk fat from mice contains about 35% MCFA). The synthesis of these milk-specific fatty acids has been used as an index for assessing the in vitro response of mammary tissue from several species to lactogenic hormones[5-8]. These studies indicate that prolactin regulates the synthesis of milk fats in a manner similar to that of other milk-specific products.

The evaluation of this and other prolactin effects is complicated by the contamination of prolactin preparations with other pituitary hormones. For example, the reported antidiuretic effect of prolactin[9,10] may be the result of its contamination with small but physiologically active amounts of vasopressin[11-13]. Likewise, the stimulation of fatty acid synthesis in WRK-1 cells by prolactin is also the result of vasopressin contamination[14,15]. Since the WRK-1 cell line was established from a rat mammary tumor induced by DMBA, this observation led to the suggestion that fatty acid synthesis and possibly other lactogenic responses of normal mammary tissue might be stimulated by this neurohypophyseal hormone[14]. Therefore, the effect of vasopressin on milk fat synthesis needs clarification.

Methods. Rat prolactin (NIH B-1; 7 IU/mg) and ovine prolactin (NIH oPRL-10, 25 IU/mg; NIH oPRL-14, 30 IU/mg) preparations were kindly supplied by the National Pituitary Agency (NIAMDD). Bovine insulin (25 U/mg), cortisol, and ovalbumin were purchased from Calbiochem (LaJolla, CA). Synthetic arginine vasopressin (160 U/mg), oxytocin (16 U/mg), and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO).

Vasopressin contamination of pituitary prolactin samples was measured by radioimmunoassay (RIA) using the method of Skowsky et al.[16]. Initial studies showed that extraction of these samples was not necessary, probably because of their relative purity. For the RIA, rabbit anti-vasopressin antiserum (Calbiochem, lot 142,124) at a final dilution of 1:80,000 in 0.08% normal rabbit serum was added to various dilutions of vasopressin or unknown samples in 200 µl of buffer (20 mM Na_2HPO_4, 0.15 M NaCl, 0.1% bovine serum albumin, and 10 mM EDTA, pH 7.4). After incubation for 24 h at 4 °C, approximately 10,000 dpm of [^{125}I]vasopressin (2200 Ci/mmole; New England Nuclear, Boston, MA) were added in 200 µl buffer. After a 2nd 24-h incubation, 600 µl of goat anti-rabbit antiserum (Sigma) in buffer was added; the 2nd antibody was used at a dilution that was previously shown to give maximum precipitation. After 2 h, all samples were centrifuged at 2000 rpm for 5 min, and the antibody-bound [^{125}I]vasopressin measured with a gamma counter. In the absence of added vasopressin approximately 28% of the labeled vasopressin was precipitated; in the presence of a large excess (100 pg/tube) of vasopressin, about 3% of the labeled material was precipitated.

Immunoreactive vasopressin was removed from ovine prolactin (oPRL-14) by treatment with rabbit anti-vasopressin antiserum. Approximately 14,000 dpm [^{125}I]vasopressin were added to 25 µg of oPRL-14 before incubation for 24 h with rabbit anti-vasopressin antiserum at a final dilution of 1:4000. Bound vasopressin was removed by adding 10 µg rabbit gamma globulin (Pentex fraction II; Miles Laboratories Inc., Elkhart, IN) and goat anti-rabbit gamma globulin antiserum.

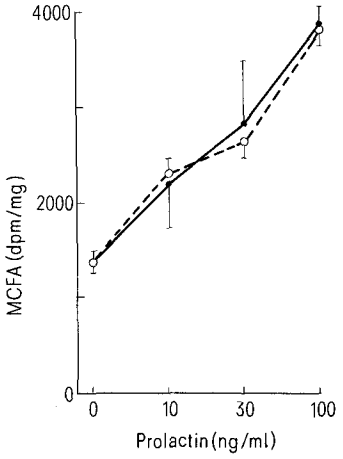
The biological activity of hormones was determined with mammary explants obtained from Balb/c mice in the middle of pregnancy. Explants were cultured as previously described[8], with the following modifications. Waymouth culture medium 752/1 (Gibco, Grand Island, NY) was buffered with 20 mM Hepes and 4 mM sodium bicarbonate (pH7.5) and gassed with air. The culture medium contained 0.1% ovalbumin or bovine serum albumin to reduce nonspecific adsorption of proteins to the cultureware. Hormones were added at the indicated concentrations.

During the last 2 h of culture, 5 µCi/ml of [^{14}C]acetate (Amersham Corp., Arlington Heights, IL; 57 mCi/mmole) were added. Tissue fatty acids were separated as their methyl esters by reverse-phase TLC[8]. The incorporation of radioactive label into medium chain fatty acids (fatty acids with a chain length shorter or equal to lauric acid) and total fatty acids was determined, and the results expressed as dpm per mg tissue. Differences between groups were analyzed using Student's t-test.

Fatty acid synthesis by mammary explants

Time	Hormone treatment		Incorporation (dpm × 10^{-3}/mg) MCFA	Total
Initial	I, F		1.9 (0.3)	51.1 (4.6)
48-h	I, F		2.5 (0.2)	81.3 (17.6)
	I, F, rPRL	(10 ng)	6.5 (0.8)***	107.7 (10.1)
	I, F, rPRL	(100 ng)	15.5 (1.7)***	135.7 (19.2)*
	I, F, rPRL	(1 µg)	19.2 (4.5)**	138.5 (17.3)*
	I, F, oPRL	(10 ng)	6.7 (0.3)***	99.9 (4.8)
	I, F, oPRL	(100 ng)	13.8 (0.3)***	111.3 (8.7)
	I, F, oPRL	(1 µg)	21.1 (3.3)***	165.7 (6.8)**
	I, F, VP	(0.1 ng)	1.9 (0.3)	65.4 (12.6)
	I, F, VP	(10 ng)	1.9 (0.2)	70.0 (9.9)
	I, F, VP	(1 µg)	1.9 (0.3)	68.3 (11.5)
	I, F, oxytocin	(1 µg)	1.9 (0.5)	73.1 (19.5)
	I, F, oxytocin	(20 µg)	1.8 (0.3)	62.3 (9.0)

Mammary explants from a 14-day pregnant mouse were cultured for 2 h (initial) or 48 h in media supplemented with insulin (I = insulin, 5 µg/ml) and cortisol (F = 1 µg/ml) and other hormones at the indicated amounts per ml (rPRL = rat prolactin, NIH B-1; pPRL = NIH oPRL-14; VP = vasopressin). Results expressed as the mean (± SEM; n = 3) in a representative experiment. Statistical comparisions on changes in fatty acid synthesis were made between the indicated group and explants cultured in I and F. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



Ovine prolactin (oPRL-14) was treated with (●——●) or without (○---○) anti-vasopressin antiserum to absorb vasopressin. The lactogenic activity of these prolactin samples was determined by adding the indicated amounts to culture medium containing insulin (5 µg/ml) and cortisol (1 µg/ml) in which mammary explants from a 14-day pregnant mouse were incubated for 48 h. The synthesis of MCFA was determined.

Results and discussion. Preparations of rat and ovine prolactin had substantial differences in their contamination with vasopressin as measured by RIA. Rat prolactin B1 contained 530 ng vasopressin per mg powder; this value compares closely to a previous report of 438 ng vasopressin per mg protein for this rat prolactin B1[14]. Ovine prolactin preparations are contaminated with much lower amounts of this peptide (Parlow, personal communication). RIA data indicated that ovine prolactin oPRL-10 had 8.2 ng vasopressin per mg powder and oPRL-14 had 1.3 ng vasopressin per mg powder. oPRL-10 has been previously reported to contain about 10 ng of vasopressin per mg protein[13].

Rat prolactin, ovine prolactin (oPRL-14), vasopressin, and oxytocin were tested for their ability to stimulate mammary fatty acid synthesis. As seen in the table, mammary explants incubated for 2 h or 48 h in culture medium containing insulin and cortisol (IF-medium) incorporated similar amounts of [^{14}C]acetate into MCFA and total fatty acids. However, the exposure of explants for 48 h to IF-medium supplemented with low concentrations (10 ng/ml) of either rat or ovine prolactin (oPRL-14) caused a significant ($p < 0.001$) increase in MCFA synthesis compared to tissue incubated in IF-medium alone. This response to prolactin was dependent upon the presence of insulin and cortisol in the culture medium; incubation of tissue in medium containing only prolactin did not increase MCFA synthesis[8]. MCFA synthesis was increased further at higher concentrations of prolactin, a maximum response occurring with approximately 100 ng/ml of either hormone preparation. Total fatty acid synthesis also increased in explants incubated with either lactogen. As seen in the table, incubation of explants in IF-medium containing 1 µg of rat or ovine prolactin (oPRL-14) caused a significant ($p < 0.05$ and $p < 0.01$, respectively) increase in incorporation compared to explants incubated in IF-medium alone. These responses to prolactin are consistent to those previously reported[8].

In contrast, explants incubated in IF-medium supplemented with vasopressin showed no significant ($p > 0.1$) stimulation of either MCFA or total fatty acid synthesis (see table). This was true at both physiological (100 pg/ml = 16 µIU/ml) and pharmacological (10 ng and 1 µg/ml) doses of this hormone[17,18]. Lower doses of vasopressin (10 pg/ml) also showed no stimulation (data not shown). Oxytocin also caused no significant ($p > 0.1$) stimulation of fatty acid synthesis by mammary explants when added to IF-medium at pharmacological (1 and 20 µg/ml or 16 and 320 mIU/ml, respectively) doses[19]. Treatment of explants with vasopressin or oxytocin appeared to decrease incorporation into total fatty acids in this experiment, but this change was not statistically significant. This reduction was not seen in other studies with these hormones. Mammary explants from pregnant Sprague-Dawley rats were also incubated for 48 h in IF-medium supplemented with ovine prolactin (oPRL-14) or neurohypophyseal hormones. The results of

these experiments were similar to those presented above for mouse explants: the addition of prolactin caused an increase in fatty acid synthesis, whereas the addition of neurohypophyseal hormones had no effect (data not shown).

The above experiments indicate that vasopressin contamination is not solely responsible for the stimulation of mammary function in vitro by prolactin preparations, since the substitution of vasopressin for prolactin does not stimulate fatty acid synthesis. In addition, prolactin preparations (rat B1 and ovine oPRL-14) with a 100-fold difference in vasopressin contamination show the same effects on MCFA synthesis at similar doses. However, these observations do not eliminate the possibility of synergy between prolactin and vasopressin.

Such interactions were examined in two ways. First, vasopressin was added to different doses of prolactin and the effect on MCFA synthesis by explants determined. Explants incubated with IF-medium and prolactin (oPRL-10; 10 an 100 ng/ml) had approximately 200% and 670% more incorporation into MCFA than explants incubated in IF-medium alone. Explants incubated with similar doses of prolactin plus vasopressin (100 pg/ml) had 220% and 610% more incorporation into MCFA than explants incubated in IF-medium alone. At each concentration of prolactin, the responses of explants incubated with or without vasopressin were not statistically different ($p > 0.1$). These results do not rule out possible synergistic interactions between vasopressin and prolactin, since oPRL-10 is contaminated with vasopressin. They suggest, however, that the effect of vasopressin must be maximal at the lowest dose of prolactin tested (10 ng oPRL-10 contains approximately 0.1 pg vasopressin).

Synergy between vasopressin and prolactin was investigated further by treating prolactin with anti-vasopressin antiserum. Excess anti-vasopressin antiserum precipitated over 85% of [^{125}I]vasopressin; treatment with additional antiserum did not precipitate more material. Treatment of ovine prolactin (oPRL-14) with excess antiserum removed 100% of the precipitable [^{125}I]vasopressin. As seen in the figure, incubation of explants with either treated or untreated prolactin caused a similar rise in MCFA synthesis.

The above experiments show that vasopressin does not directly stimulate the synthesis of milk-specific or total fatty acids in explants of mammary tissue from pregnant animals. In addition, this neurohypophyseal hormone does not synergistically interact with other hormones to affect this process. Thus, vasopressin contamination of prolactin preparations is not responsible for this lactogenic effect. A similar conclusion has been reported regarding the regulation of casein synthesis in mammary explants from pregnant rats[20]. The reason for the stimulation of fatty acid synthesis by vasopressin in the WRK-1 cell line is still unclear; however, it does not appear to reflect a differentiative process found in normal mammary tissue. The role(s), if any, that vasopressin may have on mammary gland function in vivo during lactogenesis is not addressed by this study. In addition, possible short term effects of this peptide on mammary fatty acid synthesis in vitro were not investigated.

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Normal adenylate-cyclase activity in platelets of patients with Huntington's disease

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Summary. Adenylate-cyclase activity and cyclic-AMP concentration in platelets of 7 Huntington's disease patients were found to be similar to control values.

Impaired platelet aggregation has been found in Huntington's disease patients[1]. The 2nd phase of aggregation[2] was decreased when ADP was used as an inducer, and 50% of the patients also showed disaggregation[1]. That the effect was consistent with an alteration of the release reaction was supported by reduced ^{14}C 5-HT release. However, the platelet serotonin uptake was normal[1].

We have found that the platelet response to ADP, in 4 patients, was normalized when 0,5 mM (final concentration) of sodium arachidonate was added to the platelet-rich plasma (unpublished results). This suggests that the cyclooxygenase activity is not affected in Huntington's disease.

Normally the magnitude of aggregation depends on the amount of ionized calcium and ATP available. Cyclic-AMP