globulin inhibits agglutination of erythrocytes coated with guinea pig gamma globulin when added to rabbit anti-guinea pig gamma globulin antiserum. This inhibitory effect provides a convenient, accurate, and readily interpreted technique for measurement of low concentrations of serum gamma globulin. The serum gamma globulin concentrations of germfree guinea pigs are about one-fifth those of normal guinea pigs when measured by this method, which is much lower than previously reported. The quantitative hemagglutination inhibition technique is more accurate than paper electrophoresis, and its principle is potentially applicable to measurements of many proteins or other antigenic materials.

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Stimulation of Prolactin Secretion by Perphenazine in Pituitary-Hypothalamus Organ Culture.* (28679)

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Transplantation of the anterior hypophysis into the kidney capsule of the rat maintains functional corpora lutea for prolonged periods(1,2) and initiates lactation in estrogen-primed rats(3). A similar effect resulted from infliction of a specific hypothalamic lesion in the estrogen-primed rabbit(4). Initiation of lactation was also reported following administration of chlorpromazine to women(5) and estrogen-primed rats(6) and of reserpine to estrogen-primed rabbits(7,8). Sulman and Winnik(11,12) have postulated that these effects are the result of hypothalamic depression by the above drugs, but no direct proof of this is available.

Recently Meites *et al*(13) succeeded in

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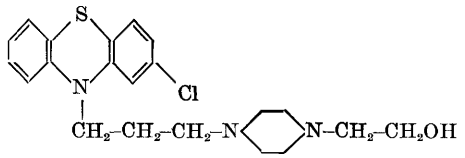
culturing *in vitro* anterior pituitary explants which secreted considerable quantities of prolactin. These quantities were increased by adding estradiol directly to the culture medium(14) or by explanting pituitaries from estradiol benzoate-primed rats(15). Pasteels (16,17) showed that addition of hypothalamic extracts or explants to the anterior pituitary organ culture decreased the quantity of prolactin released into the medium. In the work reported here we used the combined pituitary-hypothalamus organ culture to study the mechanism of action of a hypothalamic depressor on prolactin secretion *in vitro*. After comparison of various phenothiazine derivatives we chose perphenazine† as the most active prolactin releaser.



Methods. The rats serving as donors for the organ cultures were adult female rats of the Hebrew University "Sabra" strain weighing 200±20 g each, of which 50 were untreated and 50 primed with 10 mg/kg/d perphenazine for 10 days. We employed the Chen(18) method for organ culture, as described by Meites *et al*(13). After decapitation of the animals, the pituitaries and hypothalami were removed aseptically. Each of the 2 organs was cut in 6 pieces by a cataract knife, then the slices were arranged on a filter paper, placed on a raft of stainless steel mesh, and floated on 1 ml nutrient medium consisting of "199" medium to which 2 units insulin B.P.,‡ 200 U crystalline penicillin G sodium and 0.1 mg dihydrostreptomycin sulfate per ml were added. In part of the dishes the medium contained perphenazine in a concentration of 10 μg/ml.

The cultures were incubated for 7 days at 36 (± 1)°C in normal air and the nutrient medium was replaced every day, the collected medium being kept in a freezer. The media collected during 7 days of culture were pooled and assayed for prolactin in descending concentrations, by the intradermal pigeon crop-gland method (Reece-Turner, 19). The media samples were diluted until the response obtained was equal to that of 0.05 IU NIH standard prolactin solution injected on the other side of the crop. In one experiment the media were collected, kept separately every day and tested in the same manner.

† Perphenazine, 2-chloro-10-{3-[1-(2-hydroxyethyl)-4-piperazinyl] propyl}-phenothiazine was obtained through courtesy of "Taro" Ltd., Israel.

‡ Insulin proved to be unnecessary for prolactin production (author's observation and Nicoll and Meites(22)).

TABLE I

Culture type	Mean prolactin* release/day (IU)
Pituitary	.05
Pituitary + hypothalamus	.025
Pituitary + medulla oblongata	.05
Pituitary + hypothalamus from perphenazine-treated rats	.05
Pituitary + hypothalamus + 10 μg /ml perphenazine	.06
Pituitary from perphenazine-treated rats	.05
Pituitary + 10 μg/ml perphenazine	.065

* Data in the table are the mean of 3 series. Standard error of mean is about ± .01 IU.

Results. Pituitary explants cultured as described released an average of 0.05 IU prolactin daily into the medium. In fact, the output was greater in the first few days and gradually declined during the incubation period, to about one-half of the initial output at the seventh day.

Pituitary-hypothalamus co-cultures incubated under the same conditions released only 50% of the amount of prolactin produced by the pituitary alone. A control co-culture containing pituitary and brain-stem tissue released as much prolactin as the pituitary alone (Table I).

Co-cultures of normal pituitaries with hypothalami removed from perphenazine-primed rats produced the same quantities of prolactin as did pituitaries alone. Likewise, normal hypothalamus-pituitary co-cultures, when cultured on medium containing perphenazine (10 μg/ml) secreted as much prolactin as did the pituitary alone.

Thus no difference in prolactin release was

noted either with pituitaries taken from perphenazine-primed rats or with normal pituitaries cultured on a medium containing perphenazine, although in some experiments slight stimulation was observed in the latter case.

Discussion. The significance of prolactin secretion by pituitary organ-culture has been discussed by Meites *et al.*(13). The depression of prolactin production *in vitro* by addition of hypothalamus to pituitary culture has been reported by Pasteels(16,17) and confirmed by our results. These findings present direct evidence of the inhibitory role played by the hypothalamus on pituitarian prolactin secretion.

Phenothiazine tranquilizers and reserpine are known to depress certain hypothalamic functions, hence it was suggested that the signs of prolactin oversecretion resulting from their administration, *i.e.* induction of pseudopregnancy and maintenance of functional corpora lutea for long periods in rats(20,21), are also due to hypothalamic depression. The present report is, as far as we know, the first direct evidence of the mechanism of prolactin secretion initiated by perphenazine. Perphenazine, administered to rats *in vivo* or added to the nutrient medium *in vitro,* blocks the inhibitory effect of the hypothalamus on prolactin secretion. Control cultures of perphenazine-primed pituitaries or normal pituitaries cultured on perphenazine containing nutrient media did not show a significant direct effect of perphenazine on the pituitary explant.

Summary. Pituitary explants on medium "199" released an average of 0.05 IU prolactin daily. Pituitary-hypothalamus co-cultures released only an average of 0.025 IU/d. Other brain parts in co-culture failed to depress prolactin release, nor did hypothalami of perphenazine-primed rats or hypothalami to which perphenazine was added *in vitro.* The method described is a valuable tool for appraisal of prolactin releasing or inhibiting agents.

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