TABLE II. Comparison of Influenza A_2 Isolation Results Obtained from 57 Skim-Milk and from 55 Veal Infusion Broth Throat Washing Specimens, by Conventional Egg Passage and by Concentration Micro-Test.

Skim-milk throat washings*					Veal infusion throat washing				
No. of specimens	Egg passage		Concentration micro-test		No. of specimens	Egg passage		Concentration micro-test	
	Pos	Neg	Pos	Neg		Pos	Neg	Pos	Neg
57	16	41	14	43	55	12	43	12	43

* The concentration-micro-tests on skim-milk throat washings were done about 3 months after specimens were received and stored at −20°C.

ber of particles must be introduced into the egg in order to initiate infection, it means either that very few particles are infectious, or that the population as a whole is poorly adapted to the egg(4,5). It is of interest, therefore, that the progeny virus of the first egg passage also has a very low ratio of I/HA although somewhat higher than the original material. This suggests that the population is undergoing gradual adaptation to a new host manifested by the requirement for fewer viral particles to initiate infection of the egg (*i.e.*, an increasing I/HA ratio)(6).

A comparison of the direct and egg-passage tests (Table II) showed the direct method as sensitive and as reliable as the more time consuming egg-passage test. A similar direct technique has been applied to the identification of poliovirus with similar results(7).

Summary. A method of concentration and partial purification of influenza virus (A_2) found in throat washings is described. A microtechnique is presented for identification of virus contained in the concentrates.

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1. Baron, S., Proc. Soc. Exp. Biol. and Med., 1957, v95, 760.

2. Takatsy, G., Acta Microbiol. Acad. Sc. Hung., 1955, v3, 191.

3. Marmion, B. P., Curtain, C. C., Pye, J., Austr. J. Exp. Biol. and Med. Sc., 1953, v31, 505.

4. Magill, T. P., J. Exp. Med., 1951, v94, 31.

5. Liu, O. C., Henle, W., ibid., 1951, v94, 269.

6. Bernkopf, H., J. Immunol., 1950, v65, 571.

7. Peizer, L. R., Mandel, B., Weissman, D., Proc. Soc. Exp. Biol. and Med., 1961, v106, 772.

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Production of Lactation by Non-Sedative Phenothiazine Derivatives.* (29815)

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According to recent observations, the hypothalamus contains a prolactin-inhibiting factor (P.I.F.)(1,2,3), which has been shown both *in vitro* and *in vivo*. *In vitro* the hypophysis-hypothalamus organ co-culture technique(1,2,4,5) has proved that presence of the hypothalamus inhibits prolactin secretion from the hypophysis. *In vivo*, however, mammary gland development and lactation can be achieved by: (a) hypothalamic lesions in rats and rabbits(6,7), (b) cutting of the pituitary stalk in women(8) and goats(9), (c) hypophyseal autografts in rats(10) and mice (11) at sites not controlled by the hypothala-

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mus, (d) treatment with hypothalamus-depressing tranquilizers(12,13,14,15,16).

It is obvious from the foregoing that increased secretion of prolactin can be obtained either by interruption of the normal connection between pituitary and hypothalamus which frees the pituitary from the inhibitory influence of the hypothalamus, or by the paradoxical effect of depressing the P.I.F.

The present study was carried out in order to establish which chemical configuration at the phenothiazine molecule is required to obtain specific depression of the P.I.F. and thus maximal stimulation of lactation. We also sought to find out whether any correlation exists between the tranquilizing and mammotropic effects of an effective lactational agent. Finally, the aim of this study was to find phenothiazine derivatives without tranquilizing action but possessing highest mammotropic activity.

Methods. Experiments were carried out in 300 primed adult female albino rats of the Hebrew University "Sabra" strain, weighing 200 (± 10) g each. For priming, the animals received daily s.c. 8 μg estradiol in olive oil for 10 days. From the 11th day, for 5 days, *i.e.*, up to the 15th day, the drug to be tested was injected. Twenty-four hours after the last treatment (on the 16th day), all animals were sacrificed and their right inguinal mammary pad removed. The tissue was pinned on flat cork, fixed in Bouin's fluid for 24 hours and stained with hematoxylin. Evaluation of the mammotropic effect was made according to the mammotropic index (M.T.I.) presented in Fig. 1. Maximal development of the mammary glands at the end of the priming period may reach M.T.I. III. It is essential to keep strictly to a standard dose (in our rats 8 μg/d) of estradiol for priming, and to use female rats of 200 (± 10) g, because animals of higher weight or primed with higher doses may give a higher M.T.I. than III and thus yield false positive results.

Results. Table I shows that among the 14 phenothiazine derivatives tested, perphenazine (Trilafon) was the most effective in regard to mammotropic effect. Increasing the dose of perphenazine within the range 1-15 mg/kg resulted in intensified mammotropic effect. The dose of 5 mg/kg perphenazine is, however, sufficient to cause good mammary gland development in estradiol-primed rats. The following 5 preparations with a piperazine ring at N_{10} were also found quite active: perphenazine sulfoxide (15 mg/kg), fluphenazine (Prolixin, 10 mg/kg), trifluoperazine (Stelazine, 10 mg/kg), Thiopropazate (Dartal, 5 mg/kg) and butyrylperazine (Randolectil, 20 mg/kg).

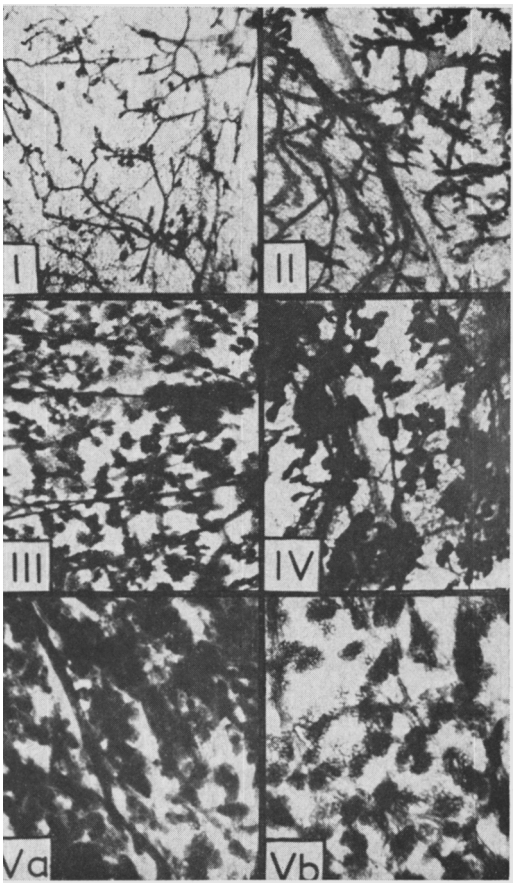


FIG. 1. Mammotropic index (M.T.I.) of mammary glands of adult female rats treated with tranquilizers, examined after fixation with Bouin's fixative and staining with hematoxylin (12 ×). M.T.I. I, II represent the effect of ineffective drugs: normal gland pattern due to estrogens and progestogens respectively. M.T.I. III, IV, V represent increasing degrees of lactogenic effects due to LTH. Va, alveoles contain milk; Vb, milk has been ejected.

Substances without piperazine substitution, as *e.g.*, thioridazine (Mellaril, 10 mg/kg) with a SCH_3 group at C_2, and chlorproma-

TABLE I. Mammotropic Effect in Estrogen-Primed Female Rats of 200 (± 10) g Weight After 5 Days' Treatment with Different Phenothiazine Derivatives.

Generic and proprietary name of drug	Daily dose (mg/kg)	No. of animals	Mammotropic index (M.T.I.) (number of animals reacting)					
			I	II	III	IV	Va	Vb
Perphenazine·HCl (Trilafon)	1	8	–	2	2	4	–	–
	5	10	–	–	–	3	5	2
	10	10	–	–	–	–	4	6
	15*	10	–	–	–	–	2	8
Perphenazine sulfoxide·HCl	15	10	–	2	–	6	2	–
Fluphenazine·HCl (Prolixin, Permitil)	10	10	–	–	–	4	6	–
Trifluoperazine·HCl (Stelazine)	10	10	–	–	–	6	4	–
Thiopropazate·HCl (Dartal)	5	10	–	–	–	3	7	–
Butyrylperazine·HCl (Randolectil)	20	10	–	–	–	8	2	–
Thioridazine·HCl (Mellaril)	10	10	–	–	4	4	2	–
Chlorpromazine·HCl (Largactil)	40*	10	–	–	5	5	–	–
Chlorpromazine sulfoxide·HCl	40	10	–	4	4	2	–	–
Dixyrazine (Esucos)·HCl	5	10	3	7	–	–	–	–
Promazine (Sparine)·HCl	40	10	2	4	4	–	–	–
Aminopromazine·HCl (Lispamol)	10	10	6	2	2	–	–	–
Levomepromazine·HCl (Nozinane)	10	6	3	1	2	–	–	–
Methopromazine·HCl (Mopazine)	10	10	2	8	–	–	–	–

* Sedative dose.

zine (Largactil, 40 mg/kg) with a Cl group at C_2, had a lesser mammotropic effect. Dixyrazine (Esucos, 5 mg/kg) which has a branched perphenazine side-chain at N_{10} and no substitution at C_2 is not mammotropic. Timeprazine (Temaril, 5 mg/kg) which is very similar to chlorpromazine has lost its mammotropic effect, apparently because of branching in the side-chain. The same holds true for aminopromazine (Lispamol, 10 mg/kg) and for levomepromazine (Nozinane, 10 mg/kg). Methopromazine (Mopazine, 10 mg/kg) is also inactive, apparently because of OCH_3 substitution at C_2.

Table III shows that neither the antidepressants, amitriptyline (Elavil), imipramine (Tofranil), nor the MAO-blockers, isocarboxazide (Marplan), iproniazid (Marsilid), administered 2 days prior to and together with perphenazine could prevent the mammotropic effect of perphenazine.

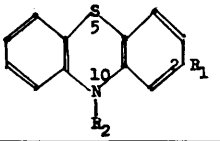
Discussion. Differences in the mammotropic effect of phenothiazine tranquilizers, ranging from M.T.I. I-Vb (Fig. 1, Table I), can be explained on the basis of their structure-activity-relationship (Table II). Substitution of H at C_2 by Cl (perphenazine, thiopropazate, chlorpromazine) or CF_3 fluphenazine, trifluoperazine) resulted in mammotropic action. Substitution of the dimethylaminopropyl group at N_{10} by a piperazinepropyl group, as in perphenazine, fluphenazine, trifluoperazine, thiopropazate and butyrylperazine, yielded increased effectiveness (Tables I & II). It seems that both the Cl and C_2 and the piperazine ring in a straight (unbranched) side-chain are essential for mammotropic activity, as is proved by the lack of mammotropic effect in promazine. Similarly, a branched side-chain, such as in aminopromazine, dixyrazine and levomepromazine, destroyed the mammotropic effect of the phenothiazine drug.

In contrast to the results of Hooper *et al* (16), we found that no parallel exists between the mammotropic and tranquilizing effects of perphenazine, as perphenazine sulfoxide and chlorpromazine sulfoxide are mammotropic but not tranquilizing(17). They caused considerable development of the mammary glands in estradiol-primed rats. Furthermore, we could not destroy the mammotropic effect of perphenazine by preceding or concurrent treatment with antidepressants such as amitriptyline (Elavil) or imipramine (Tofranil). Nor were MAO-blockers, such as isocarboxazide (Marplan) or iproniazid (Marsilid), active in preventing the mammotropic effect. Thus, it is obvious that the mammotropic and tranquilizing effects of the perphenazine congeners derive from different parts of the molecule.

Hence, it appears that the hypothalamic center which regulates prolactin secretion from the pituitary differs from the center related with sedation. This is of particular importance since it may allow stimulation of milk secretion in women and animals through hypothalamic depressants which do not affect the patients' mood. This field is at present under study.

Summary. Development of the mammary

TABLE II. Structure of the Phenothiazine Derivatives Used (Sulfoxides Contain S = O Instead of S).



Generic and proprietary name	R_1	R_2
Perphenazine (Trilafon)	Cl	$-CH_2-CH_2-CH_2-N$ (piperazine) $N-CH_2-CH_2-OH$
Fluphenazine (Prolixin, Permitil)	CF_3	$-CH_2-CH_2-CH_2-N$ (piperazine) $N-CH_2-CH_2-OH$
Trifluoperazine (Stelazine)	CF_3	$-CH_2-CH_2-CH_2-N$ (piperazine) $N-CH_3$
Thiopropazate (Dartal)	Cl	$-CH_2-CH_2-CH_2-N$ (piperazine) $N-CH_2-CH_2-O-C(=O)-CH_3$
Butyrylperazine (Randolectil)	$-C(=O)-CH(CH_2-CH_3)$	$-CH_2-CH_2-CH_2-N$ (piperazine) $N-CH_3$
Thioridazine (Mellaril)	SCH_3	$-CH_2-CH_2-$ (2-(1-methylpiperidyl))
Chlorpromazine (Largactil)	Cl	$-CH_2-CH_2-CH_2-N(CH_3)_2$
Aminopromazine (Lispamol)	H	$-CH_2-CH(N(CH_3)_2)-CH_2-N(CH_3)_2$
Dixyrazine (Esucos)	H	$-CH_2-CH(CH_3)-CH_2-N$ (piperazine) $N-CH_3-CH_2-O-CH_2-CH_2-OH$
Promazine (Sparine)	H	$-CH_2-CH_2-CH_2-N(CH_3)_2$
Levomepromazine (Nozinane)	OCH_3	$-CH_2-CH(CH_3)-CH_2-N(CH_3)_2$
Methopromazine (Mopazine)	OCH_3	$-CH_2-CH_2-CH_2-N(CH_3)_2$

TABLE III. Mammotropic Effect in Estrogen-Primed Rats of 200 (± 10) g Weight After Combined Treatment with Hypothalamic Stimulants and Perphenazine.

Drugs	Duration of treatment (d)	Daily dose (mg/kg)	No. of animals	Mammotropic index (M.T.I.) (No. of animals reacting) I	II	III	IV	Va	Vb
Amitriptyline (Elavil)	5	10	10	4	4	2	–	–	–
Amitriptyline concurrent with perphenazine	5 5	10 5	10	–	1	2	2	5	–
Amitriptyline 2 d before + 5 d concurrent with perphenazine	2 + 5 5	10 5	10	–	–	–	6	4	–
Imipramine (Tofranil)	5	12.5	8	–	1	5	2	–	–
Imipramine concurrent with perphenazine	5 5	12.5 5	10	–	1	1	6	2	–
Imipramine 2 d before + 5 d concurrent with perphenazine	2 + 5 5	12.5 5	8	–	–	6	2	–	–
Isocarboxazide (Marplan)	5	20	10	4	2	2	2	–	–
Isocarboxazide concurrent with perphenazine	5 5	20 5	10	–	2	2	4	2	–
Isocarboxazide 2 d before + 5 d concurrent with perphenazine	2 + 5 5	20 5	10	–	–	4	2	4	–
Iproniazid (Marsilid)	5	20	10	4	6	–	–	–	–
Iproniazid concurrent with perphenazine	5 5	20 5	10	–	2	2	4	2	–
Iproniazid 2 d before + 5 d concurrent with perphenazine	2 + 5 5	20 5	10	–	–	2	5	3	–

glands and initiation of lactation in estradiol-primed rats were achieved by treatment with different tranquilizers. Perphenazine (Trilafon) was found to be the most active drug. SAR studies of the phenothiazines used showed that Cl, CF_3 and SCH_3 substitution at position C_2 and a piperazine ring in the side-chain at position N_{10} of the phenothiazine molecule are essential for eliciting strong-mammotropic activity. A methoxy group at C_2 or a branched side-chain at N_{10} destroy the mammotropic effect. No parallel exists between the tranquilizing and mammotropic effects of the derivatives. Thus, the two effects derive from different parts of the phenothiazine molecule, suggesting that the mammotropic effect of perphenazine is not due to its tranquilizing properties. This became evident when a mammotropic effect was obtained with perphenazine sulfoxide and chlorpromazine sulfoxide, both substances which lack any tranquilizing effect.

We gratefully acknowledge the generous supply of perphenazine (Trilafon) and perphenazine sulfoxide from Schering, N. Y.; fluphenazine (Prolixin) from E. R. Squibb & Sons, N. Y.; trifluoperazine (Stelazine) and chlorpromazine (Largactil), chlorpromazine sulfoxide, levomepromazine (Nozinane), methopromazine (Mopazine) and aminopromazine (Lispamol) from Specia, Paris; thiopropazate (Dartal) from Searle, Chicago; butyrylperazine (Randolectil) from Bayer, Leverkusen-Wuppertal; thioridazine (Mellaril) from Sandoz, Basle; dixyrazine (Esucos) from Zori, Tel-Aviv; promazine (Sparine) from Taro, Haifa; isocarboxazide (Marplan) and iproniazid (Marsilid) from Hoffmann-La Roche, Basle; amitryptyline (Elavil) from Merck, Sharp

& Dohme, West Point, Pa.; imipramine (Tofranil) from Geigy, Basle.

1. Talwalker, P. K., Ratner, A., Meites, J., Am. J. Physiol., 1963, v205, 213.
2. Pasteels, M. J. L., C. R. Acad. Sci. (Par.), 1962, v254, 2664.
3. Gosz, H. J., Rothballer, B., Nature, 1961, v190, 349.
4. Nicoll, C. S., Meites, J., ibid., 1962, v195, 606.
5. Danon, A., Dikstein, S., Sulman, F. G., Proc. Soc. Exp. Biol. and Med., 1963, v114, 366.
6. Haun, C. K., Sawyer, C. H., Endocrinology, 1960, v67, 270.
7. Kanematsu, J. H., Sawyer, C. H., ibid., 1963, v73, 345.
8. Ehni, G., Eckles, N. E., J. Neurosurg., 1959, v16, 628.
9. Cowie, A. T., Daniel, P. M., Knaggs, C. S., Prichard, M. L., Tindal, J. S., J. Endocrinol., 1964, v28, 253.
10. Dao, T. L., Gawlak, D., Endocrinology, 1963, v72, 884.
11. Bardin, C. W., Liebelt, A. G., Liebelt, R. A., Proc. Soc. Exp. Biol. and Med., 1962, v110, 716.
12. Khazan, N., Primo, Ch., Danon, A., Assael, M., Sulman, F. G., Winnik, H. Z., Arch. int. pharmacodyn., 1962, v91, 291.
13. Sulman, F. G., Winnik, H. Z., Lancet, 1956, v270, 161.
14. Platt, H., Sears, H. T. N., ibid., 1956, v270, 401.
15. Meites, J., Proc. Soc. Exp. Biol. and Med., 1957, v96, 728.
16. Hooper, J. H., Welch, V. C., Point, P., Shackelford, R. T., J.A.M.A., 1961, v178, 506.
17. Guttman, H. N., Friedman, W., Trans. N. Y. Acad. Sci. Ser. II, 1963, v26, 75.

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Acid Mucopolysaccharides in the Skin of Lathyritic Rats.* (29816)

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Ponseti and Shepard(1) suggested that the tissue mucopolysaccharides (MPS) might be responsible for the dramatic changes produced in mesenchymal tissue by lathyrogenic agents. Several reports have since appeared concerning the concentration and metabolism of MPS in experimental lathyrism. Such reports suggest a defect of the synthesis of MPS in tissues such as epiphyseal plates and bone callus(2-5). A clear cut evidence of such a defect in other tissues is lacking.

Rat skin appears to be particularly suitable for the study of the effect of lathyrogenic agents on MPS for many reasons: Its MPS content has been extensively studied(6,7) and is known to consist of chondroitinsulphate B (ChsB), hyaluronic acid (HA) and heparin (Hep); MPS turnover is active in this tissue (8,9) and gross changes have been demonstrated in the skin of lathyritic rats(10,11). The present report is concerned with the isolation and characterization of MPS from the skin of rats treated with aminoacetonitrile (AAN), known to be the most powerful lathyrogenic agent in these animals(12).

Procedure and methods. In 5 separate studies, 50 Holtzman male rats weighing 50 to 60 g were kept on a diet of ground Purina Chow containing 0.075% AAN for 3 weeks; 50 male control animals of the same size received the same amount of food, but without AAN. The food intake of the control animals was restricted so that at the end of the 3 weeks the weight of the experimental and control animals was approximately the same. Roentgenograms were obtained at the end of the experiment to verify the degree of skeletal changes typical of lathyrism. The rats were sacrificed with ether and weighed. Immediately after death, skins were removed and stored at —20°C. At the moment of use the skins were cut into strips; hair, epidermis and subcutaneous fat were removed by dissection. The skins were then minced, dehydrated in 4 changes of acetone for 24 hours and defatted in boiling n-pentane in a Soxhlet apparatus for 2 days with 5 changes of solvent.

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