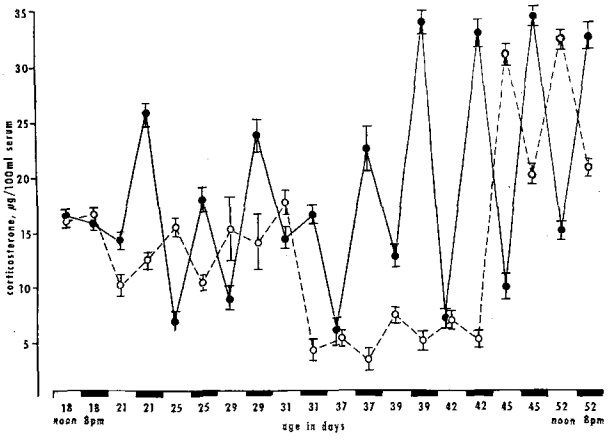
The Changes in Basal Corticosterone Secretion in Rats Blinded at Birth

Recently we have found that the onset of a daily variation in serum corticosterone is correlated in time with the development of a retinohypothalamic projection to the suprachiasmatic nucleus of the hypothalamus[1]. Light and dark have been known for a long time to influence the periodicity of a number of endocrine rhythms, including the daily cycle of corticosterone secretion[2,3]. We therefore decided to investigate whether visual input is required for the appearance of an adrenal rhythm.

Female Sprague-Dawley derived rats were obtained from Simonsen Laboratories, Gilroy, California at midgestation and were placed in a 14 h light, 10 h dark schedule (lights on at 3 h). The day after birth litters were reduced to 8 animals. The females were anesthetized with cold and half of the animals were blinded by optic enucleation. The remainder, hereafter referred to as sham-operated, were subjected to a single midline abdominal incision as a control for the stress of surgery. The animals were returned to their mothers and left undisturbed until 18 days of age. Blood samples were collected by decapitation on day 18 and day 21 at the usual time of the trough (08.00 h) and peak (16.00 h) of serum corticosterone in rats maturing under our animal room conditions[4]. The remaining litters were weaned at noon on day 21 and the weanlings placed 3 per cage. Blood samples were collected by cardiac puncture at the ages shown in the Figure. No animal was sampled more than once every 4 days. The blood was handled as previously described and corticosterone was assayed by means of a fluorometric procedure[4].



Serum corticosterone levels in sham-operated and blinded females. Samples were taken by decapitation on days 18 and 21 and by cardiac puncture on subsequent days. Each point represents mean ± standard error for 5 rats. Solid circles, intact, sham-operated females; open circles, females blinded at 1 day of age. Samples were taken at 08.00 h and 16.00 h.

The values of serum corticosterone obtained in females at different ages are shown in the Figure. At 18 days of age, the 08.00 h and 16.00 h samples were not significantly different in either intact or blinded rats. Beginning on day 21, sham-operated rats showed clear evidence of a daily rhythm (F test, $p = 0.001$), which increased in amplitude shortly after puberty (37.2 ± 1.2 days). Blind females did not show evidence of a daily rhythm until vaginal opening (45.5 ± 2.0 days). At that time (the 45 day sample) a rhythm appeared which was reversed to that of sham-operated rats. Prior to day 45, the range of serum corticosterone was first around 10–17 µg per 100 ml serum and then 2.5–7 µg per 100 ml serum.

Since only 2 sample times were chosen, it is impossible to tell whether a rhythm was absent in the blinded rats before day 45 or whether a phase shift was occurring so that by day 45 it was detectable in the 2 time periods chosen. HALBERG[2] has found that in adult mice, blinding causes a shift in both the frequency and the acrophase of the serum corticosterone rhythm so that by 3 weeks after surgery, the rhythm in blinded rats is 180° out of phase with sham-operated controls[5].

Zusammenfassung. Nachweis, dass sich bei postnatal geblendeten Ratten ein Tagesrhythmus entwickelt, der allerdings später als bei den Kontrolltieren auftritt. Der Rhythmus scheint umgekehrt zu sein.

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COGITATIONES

On the Antagonism of Ergot Alkaloids and Dopamine by Phenothiazines

Several groups have now provided evidence for an interaction between some of the alkaloids extractable from ergot (*Claviceps purpurea*) and dopamine receptors. Some of the alkaloids may directly stimulate dopamine receptors[1–3] but some are thought to antagonize the actions of dopamine on the receptors[4–6]. It would also seem likely that the phenothiazine group of compounds, which are known to be antagonists of dopamine[7–10] can also antagonize some of the actions of ergot alkaloids[11–13] including lysergic acid diethylamide (LSD) induced hallucinations[13,14].

In an effort to understand why phenothiazines should antagonize ergot alkaloids, molecular models have been constructed of D-LSD, a potent hallucinogen known to

produce a psychosis-like state in man, and of some phenothiazine derivatives chosen for their clinical efficacy against psychoses in man.

As a result the observation has been made that the phenothiazine molecule could overlap part of the LSD molecule, and this raises the possibility that these substances could act at the same receptor site. For example, in Figure 1A, ring I of the phenothiazine, in this case chlorpromazine, can occupy the position of ring C of LSD. This is made possible by the arrangement of restricting bonds around ring C which results in this cyclohexyl ring being quite rigidly fixed in space and of approximately planar configuration. As a result of the superimposition of ring I on ring C, the phenothiazine side chain can now be superimposed on part of ring D and its side chain of LSD. The electronegative chloro-substituent of chlorpromazine will be directed along one of the bonds of ring A, and we might speculate that the underlying receptor possesses a strongly electrophilic grouping in this region, helping to stabilize the active molecule by electrostatic forces. In general, the more electronegative is this ring I substituent, the more active the phenothiazine[11].

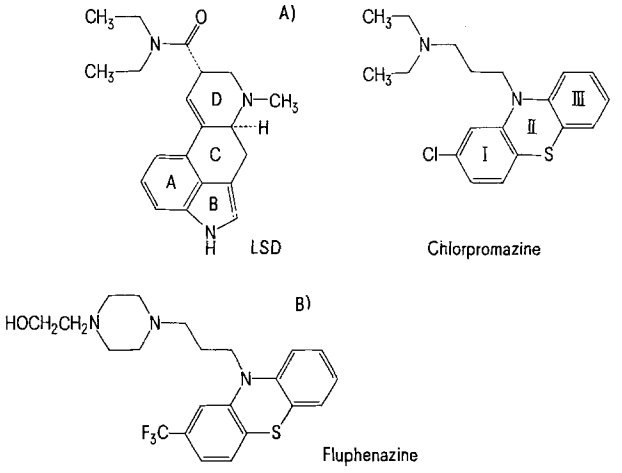

Fig. 1. Molecular structures of D-lysergic acid diethylamide (LSD), and 2 phenothiazines currently in clinical use, chlorpromazine and fluphenazine.

A particularly interesting observation about this relationship between the phenothiazine and LSD molecules is that, just as in LSD the diethylamide grouping is crucial to psychotomimetic potency and almost any alteration produces a less active hallucinogen[14] so in the phenothiazine series those compounds whose side chain can most closely approximate the diethylamide group have the greatest antipsychotic potency. Hence compounds such as fluphenazine, perphenazine and trifluoperazine which have a piperidine ring in the side chain (and a highly electronegative substituent in ring I) are the most potent antipsychotic phenothiazines in therapeutic use[11] (Figure 1B).

A further point to be made about this apparently hypothetical schema is that the phenothiazine conformation postulated here and illustrated in Figure 1A, is that which has been shown to occur for chlorpromazine by the X-ray analysis studies of HORN and SNYDER[15]. These workers also emphasize that this structure allows a superimposition of the dopamine formula on part of the chlorpromazine formula in such a way that the dopamine ring occupies the site of ring I and the side chain nitrogen atom bears a similar relationship in space to the ring. The 3-hydroxyl group of dopamine then becomes equivalent to the electron-donating sulphur atom of the phenothiazine (Figure 2).

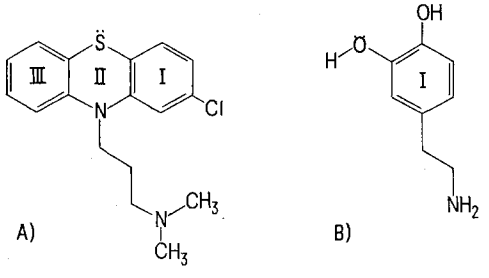

Fig. 2. Molecular structures of A) chlorpromazine and B) dopamine. According to HORN and SNYDER[15] the dopamine structure will superimpose on the known conformational structure of clorpromazine (A).

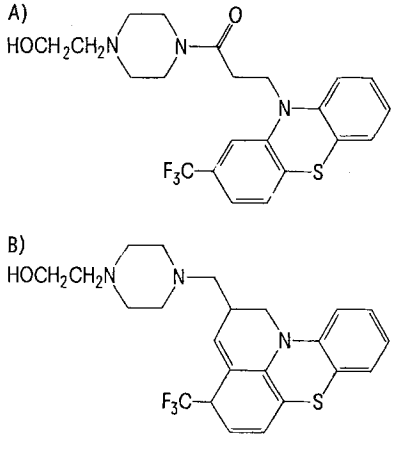

Fig. 3. Two phenothiazine derivatives which should be tested for antipsychotic and LSD antagonistic properties.

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The potential significance of such similarities of molecular structure lies in the fact that LSD for example is a potent psychotomimetic drug[14], phenothiazines are in general antipsychotic[11] and dopamine, as a probable central neurotransmitter, is being increasingly implicated in normal and abnormal behaviour patterns partly since neuroleptic potency can be correlated with dopamine antagonist potency[9,10,16–18]. If it is possible to identify a common site of action at the molecular level we may be nearer to identifying the primary cause of psychotic disorders.

The possible existence of a common receptor site for dopamine, ergot alkaloids and phenothiazines may explain some of the pharmacological interactions mentioned above, and would seem to explain some structure-activity relationships. To test the validity of these ideas it would be interesting to test a phenothiazine such as that in Figure 3A with a side chain carbonyl grouping, or that in Figure 3B with a complete 'ring D equivalent' for antipsychotic and LSD-antagonistic properties. What would be the effects of lysergic acid amide with a piperazinylethanol side chain as in fluphenazine?

Résumé. On a construit des modèles moléculaires de plusieurs phénothiazines et du LSD, et observé que la structure des phénothiazines peut se superposer à celle du LSD. Cette observation explique peut-être pourquoi les phénothiazines manifestent un antagonisme envers les alkaloïdes d'ergot. La similarité de ces structures est la plus exacte pour les phénothiazines qui combattent le plus efficacement les psychoses.

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PRO LABORATORIO

A Simplified Method of Nerve Organ Culture

The double coverslip assembly technique introduced by Maximow[1] is one of the most frequently used methods for nerve organ culture. It provides a good system for visual observation, using bright field and is also more economical in terms of feeding solution requirement than the other organ culture methods, e.g., the flying coverslip technique[2], the flask method[3], etc. However, it also has considerable disadvantages. The concave well, acting as an optical system, interferes with the use of phase contrast. The nutrient medium cannot be rapidly changed and

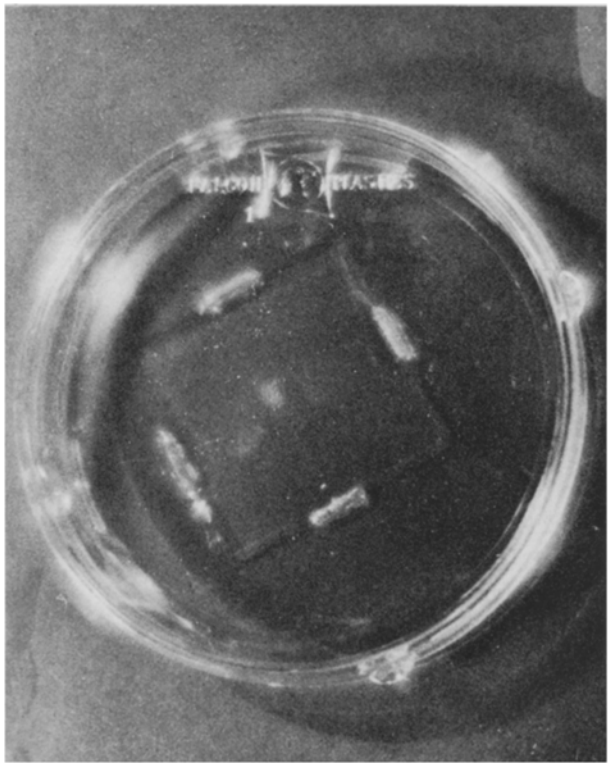

Fig. 1. Square coverslip with nerve tissue on its surface in the Falcon Plastic Petri Dish. The grooves are well seen along the side of the coverslip.

Fig. 2. Neuron and glia cells from newborn rat motor cortex, 10 days after explantation. Phase contrast. × 500.