Thus the *trans*-isomer of 2-(3,4-methylenedioxyphenyl) cyclopropylamine (MPCA) (IVa), but not the *gauche* (IVb) is active in inducing dopamine-like effects in the striatum[17]. Similarly a rigid molecule like ADTN (III), which is conformation-wise almost identical to *trans*-dopamine, has been reported to be a dopamine receptor agonist[18]. Recently HORN[19] has also provided evidence that, besides being a dopamine receptor agonist, ADTN is a potent inhibitor of dopamine uptake and thus the preferred conformation for dopamine at the uptake site should be akin to that of ADTN i.e. *trans* (O-N bond distance of 7.8Å is identical both in V and III).

But we have to keep in mind that dopamine has 2 conformational centres. One centre results in structure (V–VII) as depicted by RAKKER et al.[20] and COSTALL et al.[17] (Figure 1) while the other conformational variable depends on the rotation of the catechol ring. Thus, more precisely, the dopamine structure embedded in the ADTN molecule is certainly *trans*, but with the catechol ring *transoid* to α- and β carbons of dopamine (Va) (Figure 2). Thus, post-synaptically the preferred conformation of dopamine could be *trans-transoid* (Va). Figure 2 also depicts a topographical sketch of the complimentary post-synaptic dopamine receptor and explains how the *trans-transoid* dopamine and ADTN fit into the proposed receptor sites.

Interestingly, while ADTN elicits characteristic dopamine-like activity on specific dopaminergic neurons of the snail *Helix aspersa*, apomorphine does not[21]. This dramatic selectivity of ADTN by snail dopaminergic receptors may account for the fact that the snail receptors may resemble mammalian post-synaptic dopamine receptors and that apomorphine may not act post-synaptically, a suggestion in accordance with recent reports[8]. Structure activity studies on apomorphine analogues have made it abundantly clear that the presence of 11-OH group in correct spatial relationship to nitrogen is essential for dopamine-like activity[22]. Therefore, it seems probable that this structural requirement for apomorphine is specific and complimentary to a distinctly different pre-synaptic dopamine receptor. More precisely the dopamine conformation embedded in the apomorphine molecule is also *trans*-like (V) but with catechol ring *cisoid* to α- and β-carbons of dopamine side chain (Vb) (Figure 3). Thus, pre-synaptically the preferred dopamine conformation appears to be *trans-cisoid* (Vb). A topographical sketch of the proposed pre-synaptic dopamine receptor, on which apomorphine may interact, is also shown in Figure 3. After careful evaluation of the arguments for and against the direct action of apomorphine and ADTN on the dopamine receptors, we propose that the post-synaptic and pre-synaptic receptor of dopamine differ in the conformational restrictions they impose on agonist molecules. Rigid molecules like ADTN hold *trans-transoid*, while apomorphine incorporates *trans-cisoid* conformation of dopamine and may interact with post-synaptic and pre-synaptic receptors respectively; while dopamine by virtue of its molecular flexibility can fit in both. The approach may appear to be an over-simplification; nevertheless, the proposed hypothesis should provide a useful framework for designing new agents for further investigations.

Summary. The controversial literature reports leave open a question whether apomorphine (APO) and dopamine (DA) share a common receptor? After careful evaluation of the arguments, both for and against, about direct action of APO on DA receptor we propose that rigid molecules like APO hold *trans-cisoid* conformation and preferably interact with the pre-synaptic DA receptors while ADTN (2-amino-6,7-dihydroxy, 1,2,3,4-tetrahydronaphthalene) incorporates *trans-transoid* conformation and primarily acts on post-synaptic DA receptors. Dopamine, by virtue of its molecular flexibility, can act on both the receptors.

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The Uptake of ^{35}S by Hypothalamic and Neurohypophysial Proteins Following Intraventricular Injection of L-Cysteine-^{35}S-Hydrochloride in Rats Dehydrated and Reserpinized

There is some evidence showing that the neural input to the supraoptic and paraventricular neurones is noradrenergic as well as cholinergic, the former being inhibitory and the latter stimulatory[1]. In the hydrated rats, however, both adrenergic and cholinergic mechanisms were recently observed to provoke the release of vasopressin[2]. The data concerning changes of vasopressin liberation as influenced by reserpine-induced inhibition of monoaminergic transmission are so far not consistent: an augmentation[3,4], a decrease[5,6] and only negligible effect[7] have been reported. Reserpine has been found to inhibit the vasopressin response to hyperosmotic stimulation[8] and to diminish the block of the milk ejection reflex induced by stress[9].

The present work deals with the incorporation of ^{35}S into TCA-precipitable proteins of the hypothalamo-neurohypophysial system in white rats dehydrated and reserpinized.

Material and methods. Male rats of F_1 generation, weighing 275–350 g, bred of August males and Wistar females were used. The animals were maintained in a 14-h light,

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Mean specific activity of TCA-precipitable proteins in the hypothalamus and neurohypophysis of rats dehydrated for 48 h as influenced by reserpine treatment

Experimental group (No. of animals in parentheses)	dpm of ^{35}S-cysteine/min/μg protein (mean ± SD)		
	Hypothalamus	Neurohypophysis	H:NH[a]
Controls (7)	109 ± 23.0	15.6 ± 5.8	7.0
Rats injected with reserpine 24 h before killing (9)	80.5 ± 19.2	9.9 ± 2.5	8.1
Significance (Student's t-test)	$p < 0.02$	$p < 0.05$	

[a] H, hypothalamus; NH, neurohypophysis.

10-h dark cycle (lights on from 06.00 h–20.00 h). Before the experiment they had free access to standard rat pellets and water ad libitum. During 2 days preceeding experiment, the animals were deprived of water and received rat pellets only. They were slightly anaesthetized with ether, immobilized in a simple stereotaxic apparatus and injected into the left lateral cerebral ventricle with 10 μCi/100 g of initial body weight of L-cysteine-^{35}S-hydrochloride (The Radiochemical Centre, Amersham, batches No 08143, spec. act. 42.0 mCi/mmol and No 29163, spec. act. 33.5 mCi/mmol, dissolved with isotonic saline). The volume injected was about 20–25 μl, the duration of the injection about 10–15 sec. In a few pilot experiments, the stereotaxic coordinates were proved to be suitable for intraventricular injection in rats of the same strain and similar body weight. Since a circadian rhythm of incorporation of radioactive precursors into cerebral proteins has been recently reported[10], all the injections of labelled material were executed between noon and 14.00 h.

3 h after the isotope administration, the rats were decapitated; the brain with intact pituitary was quickly removed, the pituitary stalk cut just on the adenohypophysial border and the neurohypophysis separated under stereomicroscope. From the brain, the hypothalamus was dissected and weighed. In order to receive the TCA precipitate, tissue samples were treated following the procedure recommended by TER HAAR and MACKINNON[11]. After drying, the precipitate was dissolved in 0.5 ml of 0.45 M NaOH at 90 °C. A sample (100 μl) of the NaOH solution was taken for determination of protein by the method of Folin-Ciocalteu as modified by LOWRY et al.[12]. Human serum protein (Serostandard, batch No 101272, BIOMED, Cracow) was used as standard.

Following MAHIN and LOFBERG[13], 200 μl of the NaOH solution was added to 5.0 ml of ethyleneglycol monoethyl ether (Cellosolve Solvent, Koch Light) and 10.0 ml of a scintillation fluid (6.0 g of 2,5-diphenyloxazole, Fluka AG, per 1 l of toluene). The radioactivity of this mixture was estimated in an automatic LKB-Wallac liquid scintillation counter, type 81000, for 20 min. Finally the desintegrations per min of ^{35}S-cysteine which had been incorporated per 1 μg of protein were calculated.

Complete experimental procedure has been carried out on 16 rats divided into 2 groups: A) 7 controls, i.e. animals simply dehydrated for 48 h; B) 9 rats similarly dehydrated and additionally injected i.p. with reserpine, 10 mg/kg of initial body weight, after 1 day of water deprivation, i.e. 24 h before isotope administration.

Results and discussion. In animals dehydrated for 48 h, the mean specific activity of hypothalamic and neurohypophysial TCA-precipitable proteins appears to be significantly higher as compared with that found in animals dehydrated and reserpinized (Table). The ^{35}S-amino acids uptake in the hypothalamo-neurohypophysial system is known to be closely related to the synthesis of neurohypophysial principles[14–19]. It follows, therefore, that the inhibition of monoaminergic transmission, as caused by reserpine in dehydrated animals, does result in an impairement of the synthesis rate of neurohypophysial hormones and/or their carrier proteins. This seems to be consistent with some previous observations on reserpine-induced inhibition of the antidiuretic response during dehydration[5,6] and hyperosmotic stimulation[8].

The relation of mean specific activity of TCA-precipitable proteins in the hypothalamus to that in the neurohypophysis, as found in reserpinized animals, is not significantly different from that in the controls (Table). So it appears that the axonal transport of freshly synthetized neurohormones and/or neurophysins is not seriously affected by reserpine. A reduction of the vasopressin outflow from the cut infundibular stalk has been noted, however, in reserpinized but not dehydrated rats[20].

Under physiological conditions, the synthesis, transport and release of neurohypophysial hormones are thought to change each in the same direction, i.e. increased release should be accompanied by enhanced synthesis as well as transport rates, and vice versa. The augmentation of the synthesis[17,21], transport[19,22], and turnover[19] of neurohypophysial peptides, all brought about by osmodetector stimulation during dehydration, are here a good example. But in the light of recent work done in this laboratory, there should be some doubt concerning similar parallelism in dehydrated and reserpinized rats. The present results indicate the existence of a reserpine-sensitive monoaminergic mechanism stimulating – or, at least – maintaining the biosynthesis of neurohypophysial hormones in dehydrated animals. Nevertheless, the depletion of

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vasopressin and oxytocin stores in hypothalamus and neurohypophysis of rats deprived of water up to 12 days was found either not to change or even to be more marked under reserpine treatment[23]. It may therefore be concluded that biosynthesis and release of neurohypophysial hormones in dehydrated animals seem to be influenced by reserpine each in a different way. The hypothetical assumption is that the existence of at least two kinds of reserpine-sensitive supraoptic and paraventricular afferents of monoaminergic origin cannot be excluded – one stimulating the rate of neurohormone synthesis and the other inhibiting its release[24, 25].

Summary. In rats dehydrated for 48 h and injected intraventricularly with L-cysteine-^{35}S-hydrochloride, the specific activity of TCA-precipitable material, both in the hypothalamus and neurohypophysis, was found to diminish under reserpine treatment.

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The Site of Triglyceride Biosynthesis in Milk

The study of triglyceride biosynthesis by the mammary gland using biopsy samples of mammary tissue in vitro is beset by numerous practical difficulties, not the least of which is the removal of the large amount of fibrous connective tissue in the gland. In 1964, McCarthy and Patton[1] found that freshly-secreted goat milk would actively incorporate isotopically-labelled fatty acids into lipids on incubation in vitro. Recently the potential of this simple system for the study of triglyceride biosynthesis in vitro was investigated and it was determined[2] that this activity was enzymatic and that the enzymes of both the glycerol-3-phosphate and monoglyceride pathways of triglyceride biosynthesis were present. Also, stearic acid was desaturated to oleic acid in milk and virtually all the oleic acid formed was found in esterified form. This, together with the finding that the reactions were not stimulated by additions of the relevant cofactors (ATP, glycerol-3-phosphate, coenzyme A and Mg^{++} for glyceride synthesis, together with NADH for desaturation) singly or in combination suggested that the enzymes concerned were contained within a membrane-bound particle in goat milk. Patton, Durdan and McCarthy[3] localized the active fraction in skim milk by centrifuging it after the incorporation of radioactivity from added fatty acids. Nearly all of the radioactivity was found in a fraction immediately above the casein pellet which contained a loose fluffy material. Freshly-secreted sow's milk (expressed after oxytocin administration) which had less than half the biosynthetic activity of goat milk, showed a similar localization[4]. The material was not characterized morphologically in these studies but it was suggested that it was the lipoprotein material known to be present in skim milk. This material had been called 'milk microsomes' by Bailie and Morton[5] because of its biochemical similarity to tissue microsomes. Recent work has indicated several possible sources for the lipoprotein fraction from skim milk, notably the milk fat globule membrane[6], the secretory cell plasmalemma[7], and debris from leucocytes[8]. In non-mastitic milk, the first two probably predominate. The present report describes a simple method for the isolation of the active fraction from goat skim milk and demonstrates that the material synthesizing triglyceride consists not of 'milk microsomes' but mainly if not exclusively of plasma membrane bounded pieces of cytoplasmic material from the secretory cell.

Freshly-secreted goat milk[2] was centrifuged at 40,000 *g* for 15 min in an angle-head rotor on a MSE High Speed 25 centrifuge at 0 °C. The skim milk fraction was removed from the sedimented material and added to 5 volumes of 0.08 *M* calcium chloride solution. A pellet, formed on centrifugation at 400 *g* or for 5 min at 4 °C was washed once with 10 ml of 0.15 *M* KCl solution buffered with 0.1 *M* phosphate (pH, 7.0, 4 °C) and was then suspended in this solution for incubation in vitro with (1-^{14}C)-palmitic acid as described earlier[2]. (Calcium chloride solutions have been used in a similar manner to isolate microsomes from cellular homogenates[9]). The pellet had 80–150 % of the biosynthetic activity of an equivalent volume of 40,000 *g* skim milk.

Examination of the pellet by electron microscopy showed that it consisted mainly of membrane-bounded pieces of cytoplasmic material each with a lipid droplet but no nucleus (Figure 1). Rough endoplasmic reticulum in sheets, vesicles or swollen cisternae was usually present in the pieces with an occasional mitochondrion (Figures 2 and 3). More rarely parts of the golgi apparatus could be identified, together with granule-containing vesicles which were identical to the golgi vesicles containing casein that are typical of the lactating mammary secretory epithelial cell. On any one section the cytoplasmic pieces varied considerably in the degree of structural preservation of the organelles and could be arranged in a hypothetical sequence of degeneration (Figure 1).

No sign of any of the vesicular structures characteristic of the skim milk lipoprotein[6, 7] was found in the precipitate from calcium-treated skim milk. Autoradiography of sections of the pellet of the precipitated material, which

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