The 3 physical examinations, namely maximum absorption spectroscopy (MAS), IR- and mass spectroscopy (MS) were made with the methanol extract only. The ethanol extract was examined for maximum absorption spectroscopy.

Results. Chemical: The deep yellow color of the solution spots was changed to pink by the applied acids. Concentrated nitric acid produced a pink periphery but the centre was stained distinctly blue. Tests for bilirubin, urobilin and for urobilinogen were negative. The pigment was soluble in water, methanol, ethanol and especially in amyl alcohol. There were no differences in the outcome of the described tests between the methanol and the ethanol extracts.

Physical: The MAS of the alkaline fluid was 432 nm and after acidification 476 nm. The IR-spectrum showed CO- and NH-groups; the mass spectrum showed a peak at 229.

Discussion. The analytical procedures proved the presence of a peculiar pigment in only 4 pure uric acid stones and not in the 14 other pure tri-oxy-purine calculi or in the 150 oxalate-phosphate concretions. The 4 patients who had been hospitalized for stone operation had not been treated with color-producing drugs or fed with large amounts of specific food which might have imparted the stain to the concretions. One must, therefore, assume that this peculiar pigment originated from metabolic processes in the human body and was excreted by the kidneys.

Our first assumption[2] that this urinary natural dye in the calculi might be urorosein was based on 3 facts: 1. the easy solubility in amyl alcohol, 2. its pink color in an acid medium and its amber yellow in an alkaline, 3. its property to stain crystals of uric acid pink. Urorosein is a polymer of an α-β-di-indolyl methene (VON DOBENECK[4]). Its MAS of 535 nm, as well as its molecular weight, are against the idea that our pigment is a derivative of indole.

The mass spectrum of 229 excludes all tetrapyrroles (bilirubin, urobilin) as the staining agent. On the other hand, a molecular weight of 229 and the presence of CO and of NH-groups shown by the IR-spectrum might be consistent with the assumption that our pigment could be a di-pyrrole; more specifically of the Neo-type (an oxo-di-pyrrole methene with at least 1 vinyl group (VON DOBENECK[5]). Compounds of such chemical configuration show spectroscopically a shift to higher absorption maxima in an acid milieu than in an alkaline, as has been shown by NICHOL and MORELL[6] for certain bilirubin derivatives. Such a shift is also characteristic for the pigment described here.

There are other di-pyrroles in normal and pathological urines. WITH[7] mentiones urochrome-B, the fuscins and the leukans. Meso-bilifuscin, a di-pyrrole, a brown pigment has been described by MORAVEC[8] to be soluble in glacial acetic acid. However, these di-pyrroles, as well as the pent-dyo-pent and pro-pent-dyo-pent compounds, present quite other chemical and physical properties than does our stone pigment (VON DOBENECK[9]).

Zusammenfassung. Es wird das Vorkommen eines bisher unbekannten Pigmentes in Harnsäuresteinen beschrieben. Chemisch handelt es sich wahrscheinlich um eine Oxo-di-pyrrol-methen-Verbindung.

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3,4-Dihydroxyphenylacetic acid (DOPAC): a Possible Endogenous Inhibitor of Indoleamine-N-Methylation in the Rat Brain

In recent years, a number of important observations concerning the occurrence and properties of an indoleamine- and catecholamine-N-methylating enzyme in mammalian brain have been reported[1–4]. It seems to be generally accepted that 5-methyltetrahydrofolic acid serves as the methyl donor for this enzymatic reaction. Several N-methylated monoamines have been implicated in the aetiology of psychotic disorders[1,5,6]. Assuming that this hypothesis is valid and that neuroleptic drugs exert their beneficial effect on psychotic disorders by interfering with these substances, there are at least 4 possible ways in which they may act: a) they may inhibit the enzyme directly, b) they may block the receptors on which N-methylated amines possibly act, c) they may stimulate the production or release of endogenous substances which inhibit the enzyme, and d) they may influence the degradation of N-methylated amines.

The aim of the present investigation was to study the effects on indoleamine-N-methylation of 2 well-established neuroleptics and of the 2 endogenous amine metabolites whose concentrations in the brain are most spectacularly increased by the former[7,8].

The enzyme was prepared according to the procedure described by HSU and MANDELL[2], and LADURON[1], the whole brains of 50 male Sprague-Dawley albino rats weighing 200–250 g being used for each batch. The brains

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were homogenized in twice their volume of double-distilled water and centrifuged for 20 min at 30,000 *g* at 4 °C. The supernatant was ultracentrifuged for 90 min at 100,000 *g* and then partially purified by ammonium-sulphate fractionation. The precipitate obtained at 40–50% saturation was separated by centrifugation at 55,000 *g* for 20 min at 4 °C and then resuspended in 5 ml of 0.02 *M* K-phosphate buffer, pH 6.8. This suspension was dialyzed overnight against the same buffer and had a final volume of 8–9 ml, containing 5–10 mg protein/ml, with little variation from batch to batch. The protein content was measured according to the method of WARBURG[9].

The assay procedure was as described hy HSU and MANDELL[2], except that mercaptoethanol (1.25×10^{-5} *M*) was used as an antioxidant and the toluene-isoamyl-alcohol extract was evaporated under vacuum at room temperature overnight and the residue taken up directly into scintillator solution. The concentrations of the various reactants were as follows: tryptamine 10^{-3} *M*; 5-methyl-tetrahydrofolate (5-MTHF)-C^{14} 3.16×10^{-5} *M* (0.95 μCi per assay); K-phosphate buffer pH 6.5 (phosphate concentration 0.15 *M*); mercaptoethanol 1.25×10^{-5} *M*; EDTA 5×10^{-4} *M*; aliquots of enzyme solution containing 0.5 mg protein per assay. The total volume of each incubation mixture was 0.5 ml and the incubation time 2 h. The solutions of tryptamine, chlorpromazine, clozapine, 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxy-4-hydroxyphenylacetic acid (HVA) were adjusted to pH 6.5 prior to their addition to the incubation mixture.

The amines were extracted from the incubation medium after alkalinization. Then the organic phase was completely removed and the medium was acidified with 0.3 ml 10 *N* HCl, 0.5 g KCl was added and acidic compounds extracted with 5 ml butyl acetate[10]. To 3ml of the organic extract, 10 ml of scintillator fluid was added. The enzyme batches methylated tryptamine to N-methyltryptamine at rates ranging from 15 to 22 pmol h^{-1}/mg protein. The K_m value for tryptamine methylation was 8.6×10^{-3} *M*, which is in agreement with the findings of HSU and MANDELL[2]. The 2 neuroleptics used, chlorpromazine and clozapine ,had no influence on tryptamine methylation up to concentrations of 10^{-4} *M* and 10^{-3} *M*, respectively (Figure). Higher concentrations produced first opalescence and then flocculation of the incubation mixture, probably due to precipitation of the enzyme. The results obtained at such concentrations therefore cannot be interpreted as real signs of enzyme inhibition. It must consequently be assumed that neuroleptic drugs as such do not influence enzyme activity, because concentrations which produce unspecific decreases in N-methylation are unlikely to be attained by in vivo administration, even in animal experiments.

DOPAC and its 3-*O*-methylated analogue, HVA, inhibited tryptamine methylation to varying degrees depending on the concentration applied (Figure). The inhibitory capacity of DOPAC was 2–3 times greater than that of HVA. In the experiments in which DOPAC was used as an inhibitor, a radioactive product could be extracted with butyl acetate from the acidified reaction mixture (Table). The radioactivity first increased, then decreased with increasing DOPAC concentrations. No radioactivity was extracted in control experiments, or when HVA was used as an inhibitor. It is possible that the radioactive product formed with DOPAC is either HVA or iso-HVA. Preliminary experiments with DOPAC as substrate added to the incubation mixture showed the kinetic pattern commonly associated with substrate inhibition. This might explain the decrease in extracted radioactivity at high DOPAC concentrations in the inhibition experiments shown in the Table. This implies that the enzyme which N-methylates tryptamine is also able to *O*-methylate phenolic hydroxy groups. In fact, BANERJEE and SNYDER[4] observed *O*-methylation of 5-hydroxyindoles rather than N-methylation.

The concentrations of DOPAC and HVA needed to inhibit the enzyme are very high. However, ANDÉN et al.[11] have found the dopamine concentration in striatal nerve endings to be about 8 mg/g. A comparison of the dopamine[12] and DOPAC[8,13] contents of mammalian

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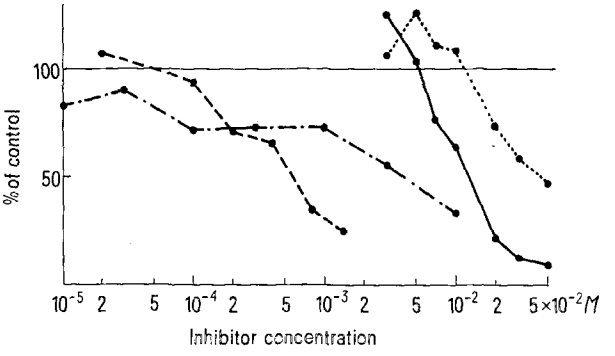
Incorporation of radioactivity into acidic substances

Concentration of inhibitor (*M*)	cpm extracted with DOPAC as inhibitor	cpm extracted with HVA as inhibitor
5×10^{-2}	5437	1776
3×10^{-2}	6836	1665
2×10^{-2}	6533	1465
10^{-2}	10136	1536
7×10^{-3}	6432	1723
5×10^{-3}	5996	1559
3×10^{-3}	4956	1486
Controls ($n = 4$)	1928 ± 76	1928 ± 76

Extraction of acidic radioactively labelled reaction products from the incubation mixture used in the inhibition experiments represented in the Figure. Each figure is the average of 2 estimations. Incubations with tryptamine alone served as controls.



Tryptamine N-methylation by a rat brain enzyme: inhibition by neuroleptics and acidic dopamine metabolites. Effects of different concentrations of chlorpromazine –––, clozapine (–·–·–·–), DOPAC (————), and HVA (----------). Activity is given in percent of activity with tryptamine alone. Each point represents the average of 2 incubations. Blanks run with boiled enzyme showed that non-enzymatic production of N-methyltryptamine was about 30% of the overall reaction. Activities of control samples ranged from 15 to 22 pmol h^{-1}/mg protein for the different batches of enzyme used.

striatal tissue shows the latter to be 5–10% of the former. As DOPAC is presumably an intraneuronal metabolite[13], its concentration in striatal dopaminergic nerve endings would be 400–800 μg/g, which is roughly equivalent to $2–4 \times 10^{-3}$ *M*, if 1 g of wet tissue is set equal to 1 ml of liquid. This concentration coincides with the threshold inhibitory concentration of DOPAC shown in the Figure.

Considering that neuroleptics increase the concentration of endogenous DOPAC enormously, it seems possible that the antipsychotic activity of these drugs is related to the rise in the intraneuronal content of DOPAC acting as a methylation regulator. This implies that the methyltransferase is localized intraneuronally. The calculation above is based on data from striatal dopaminergic neurones. There are no corresponding data available on other central dopaminergic neurones, but also no evidence to indicate that they differ very much in this respect.

Thus, the regulation mechanism outlined above is not necessarily restricted to striatal neurones. High concentrations of 5-MTHF and high activity of tryptamine-N-methyltransferase were in fact detected in rat corpus striatum, but considerable amounts and activities have also been found in other brain regions[14,15]. Tryptamine is certainly not the only substrate of this enzyme leading to the formation of psychotomimetic derivatives. Other indoleamines, for instance, such as bufotenin or 5-methoxy-N,N-dimethyltryptamine[6], and catecholamines[1] also, have been held to be possible psychotogenic substances.

It is not yet definitely known whether biogenic amines do in fact serve as 'precursors' of endogenous psychotogenic substances, much less which particular amine might be specifically concerned. Should dopaminergic neurones be involved, then it may be assumed that DOPAC fulfils a regulatory function.

Résumé. L'acide dihydroxyphénylacétique (DOPAC) et l'acide homovanillique (HVA) inhibent la méthylation de la tryptamine par une méthylase partiellement purifiée du cerveau de rat. Les concentrations nécessaires à cette inhibition sont hautes et du même ordre de grandeur que celles qu'on peut attendre du métabolisme de la dopamine au niveau des terminaisons dopaminergiques. La chlorpromazine et la clozapine n'influencent pas cette activité enzymatique.

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Feto-Specific Features of Human β_2-Microglobulin

Ontogenetic studies on plasma proteins showed that the concentration of individual proteins gradually increases with the maturation of the fetus. Some proteins reach the adult levels already during the intrauterine life, the concentration of others raises slowly and the adult levels are attained later in the extrauterine life. An exceptional developmental pattern is presented by feto-specific proteins[1] (e.g. α-fetoprotein, fetuin) that reach the highest concentrations already during the fetal period and the levels attained are higher than those found in healthy adults. Some of these proteins almost disappear from the sera after birth and can only be detected by highly sensitive techniques such as radioimmunoassay.

We have studied the ontogenesis of human β_2-microglobulin, a low-molecular-weight constituent of human serum and other body fluids[2]. This protein has been shown to be homologous to the constant domains of immunoglobulin G light and heavy chains[3], and to be produced in vitro by lymphoid cells as well as by a variety of cells derived from non-lymphoid solid tissue lines[4–7]. The present study demonstrates the presence of β_2-microglobulin in fetal sera and other fetal fluids; the concentration changes of this protein in serum during fetal development were found to be similar to those of feto-specific proteins.

Materials and methods. Human fetuses of 16 to 36 weeks of gestation were obtained from spontaneous or Cesarean abortions; the gestational ages were estimated according to the last menstrual date, the body weight and the crown-heel length. Fetal urine was obtained by needle aspiration after exposure of the bladder by dissection. Amniotic fluids were taken in connection with diagnostic amniocenteses. All samples were kept frozen at −20 °C until used.

β_2-Microglobulin was quantitated by the radioactive single radial immunodiffusion method as described earlier[8]; antisera against β_2-microglobulin were raised in rabbits by immunization with purified antigen isolated from urine of kidney transplant patients[9].

Results and discussion. β_2-Microglobulin was detected in all fetuses investigated. The concentration of the protein in fetal sera was found to be considerably higher than in normal adult sera; the mean value for the fetal sera studied was 0.71 mg/100 ml (range 0.28 to 1.36 mg/100 ml) as compared to adult serum values ranging from 0.11 to 0.24 mg/100 ml (ref. [8]). The change in β_2-microglobulin concentration with the gestational age is shown in the Figure. The highest concentrations were found in fetuses between the 20th and 32nd weeks of gestation; at the end of the second trimester the level of β_2-microglobulin was approximately 8 times higher than the mean concentration found in sera of healthy adults. After this period, the concentration of the protein decreases to reach the levels of cord blood sera (range 0.20 to 0.48 mg/100 ml). Relatively high concentrations of β_2-microglobulin were also found in fetal urines and amniotic fluids. In 5 urine specimens from 16- to 36-week-old fetuses the β_2-micro-

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