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THE FUNCTION OF IRON IN THE FORMATION OF TREMOR*

Taylor et al showed recently that α-α dipyrydil (D) when injected into rats inhibited tyrosine-hydroxylase (TH) in the suprarenal gland and that it reduces noradrenaline in the brain (41). The same authors noticed that rats which received D developed tremor.

This result interested us for many reasons. We have shown that oxotremorine (OT) significantly reduces the concentration of total iron (TFe) in the brain (17) and that the greatest reduction is in the corpus striatum (CS). We have also shown that the reduction of iron takes place in the mitochondria of the CS.

In addition to this we proved that the total flavine (TFl) in the complete brain of rats is reduced (20), as it is in the CS and the isolated mitochondria of the CS (40)

As the inhibition of TH prevents the production of DOPA, i. e. dopamine (46) and the function of this enzyme depends on iron (46, 26), the question is whether the tremor described by Taylor et al is a good model for Parkinson's disease, because it is known that the reduction of dopamine in the CS appears in the disease (25). Apart from that, D could give us a better comprehension of the role of iron at the beginning of Parkinson's tremor. So the question is: is the reduction of iron the cause or consequence of tremor.

It is known that TH is found in higher concentration in the nucleus niger (6) (NN). Therefore it was necessary to determine the iron in NN in tremor caused by D and OT. Apart from that, in this work we were interested in the influence of D on the quantity of acetylcholine (Ach) and dopamine in the brain of rat.

We also examined the influence of different drugs on tremor caused by D. We gave D locally in the globus pallidus and NN by means of steriotactic apparatus to see if the local reduction of iron in these two regions leads to tremor. As well as TFe we determined TFl and copper in the brain of rat because we have shown that tremorine reduces copper too (18). We also examined the effect of D on temperature, sense of pain and blood sugar.

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METHODS

Determination of iron in NN.

White rats of both sexes with a weight of 200—250 g. were used in the experiment. Oxotremorine (0,5 mg/kg) and D (25 mg/kg) were injected intravenously. 30 minutes after the administration of oxotremorine or D the animals were killed by anaesthesis with ether. The brain was washed out with normal saline in the following way: the thorax was opened, the abdominal blood vessels tied and the aorta ascendens in which a cannular was introduced for rinsing with normal saline was cut through. After that the vena cava was also cut through and the brain was rinsed until a clear fluid came out.

The brain was then taken out and after the removal of the small brain, it was cooled to —4°C. The cooled brain was isolated using the method of Popov et al (31). The NN, which is in region 8 (Tegmentum), is clearly differentiated from the rest of the brain mass and by using a magnifying glass with a fine occulistic scalple everything except the NN in which Fe was determined, was removed so that the tissue was homogenous, and the iron was determined spectrometrically (7). TFl and copper in CNS we determined by means of Bessey's (3) method in addion to Heilmeyer et al (21).

Statistical analysis of the results was done by the method of sequential analysis.

Dopamine was determined spectrofluometrically with Carlson and Waldeck's method (5). Ach was extracted by McIntosh and Perrys method (26) and the rectus abdominalis of frog was measured.

Of drugs we gave atropine sulphate, caramiphen, ethyl benztropine, penicillamine and alfa-methyl dopa, which inhibit intentional tremor (38), alfa-methyl-meta-tyrozine a depletor of dopamine (47), gamma-hydroxybutyrate which increases dopamine in CNS (10), and DL-4-chloramphetamine a depletor of serotonine (9). In addition we determined the activity of cholinesterase in vitro and in vivo by Michelson's method (27), 30, 60, and 180 mins after giving D in the brain and erythrocites.

By means of steriotactic apparatus (4), using Groot's atlas (14), we injected D in a dose of 1 or 10 gamma (in 2 mm) by means of »Agla« syringe directly into the globus pallidus, nucleus caudatus and nucleus niger and observed if tremor developed. The next day the animals were killed, the brains fixed in formalin and microscopic control of the position of the needle of the steriotactic apparatus was carried out. We measured tremor in the occulus which was observed always by the same technical assistant and two assistents who did not know the groups gave the estimation of tremor and compared it with that given by the technical assistent.

Because a static tremor caused by OT causes analgesis (8) and reduction of temperature (8) as well as an increase of blood sugar (16) we also examined analgesia by means of a clamp on the tail. Control rats in 3—4 secs. tried to remove the clamp with their lips, became agitated and revolved. In the analgezic effect, the trying to remove the clamp developed later or not at all, and the animals were more peaceful. We also determined the rectal temperature and the blood sugar level using the method of Gubler et al. (15) after giving D.

RESULTS

First of all we must emphasize that we noticed D (100 mg/kg i. p.) causes static tremor as well as OT. This tremor only appears after 30—60 mins and lasts 6—7 hours and sometimes longer. Any way, much longer than OT tremor.

Table I

THE EFFECT OF D (100 mg/kg i. p.) ON TFe, TFl AND Cu In THE BRAIN OF RAT IN γ/g

(8 animals in each group)

EXPERIMENTS

	Control	30′		60′		90′		120′	
TFe	82,6	60,3	p<0,005	70,7	p<0,005	86,3	p<0,01	96,8	p<0,005
TFl	2,430	2,513	n. s.	1,833	p<0,005	2,071	p<0,005	2,360	n. s.
Cu	4,30	2,00	p<0,005	2,42	p<0,005	3,51	p<0,005	4,40	n. s.

Values are expressed as γ/g tissue.
Subst.: α, α′ — dipyridyl
Dose: 100 mg/kg i. p.
Tissue: brain

As we can see from table I, D leads to a significant, reduction of the concentration TFe after 30, 60, 90, 120 mins., TFl is significantly reduced after 60 and 90 mins., and Cu after 30, 60 and 90 mins. D signi-

Table II

TFe IN SUBSTANTIA NIGRA OF RAT'S BRAIN TREATED PREVIOUSLY WITH αα DIPYRIDYL* AND OXOTREMORINE

Control	D		OT	
96,95	66,86	p<0,005	88,07	p<0,005
C	D_1	p C:D_1	D_2	p C:D_2

* Dipyridyl 100 mg/kg i. p.; OT 0,25 mg/kg i. v.; the animals were killed 30 min. after application of drugs.

Table III

THE EFFECT OF DIPYRIDYL (100 mg/kg i. p.) ON THE LEVEL OF TOTAL ACETYLCHOLINE IN THE BRAIN OF RAT

Dipyridyl was given i. p. (100 mg/kg). The values are expressed as γ/gr fresh tissue.

N. S. is not significant.

	№ of animals	Acetylcholine	change in % of control values	p
Control	7	2,65±0,08	—	—
1h	4	3,13±0,18	+18	<0,01
2h	6	3,30±0,09	+24	<0,001
3h	5	3,48±0,14	+31	<0,001
4h	4	3,22±0,11	+21	<0,01
12h	6	2,74±0,08	+ 3	N. S.
24h	4	2,67±0,17	+ 1	N. S.

Table IV

SUBSTANCE	Time of application of substance	Dosis mg/kg	Effect
Atropine sulfat	15	10 i. p.	++
„	15	20 i. p.	—
Methylatropine nitrate	15	20 i. p.	++
Caramiphen	15	10 i. p.	++
„	15	10 i. p.	—
Ethylbenztropine	15	1 i. p.	—
Penicillamine	15	50 i. p.	+++
α-methyl-dopa	4h	100 i. p.	+++
α-methyl-meta-tyrozin	4h	100 i. p.	++
DL-4-chlor-amphetamin	4h	10 i. v.	++
γ-hydroxybutirat	60′	500 i. v.	++
L-Dopa	60′	100 i. v.	++
5-hydroxytryptophane	60′	100 i. p.	++
Control	—	—	++

ficantly reduces TFe in the NN of rat (Table II). From table III we see that D significantly increases ACh 2, 3 and 4 hours after application. After 24 hours ACh returns to normal.

From table IV it can be seen that known cholinolytic-antiparkinsonitics (atropine, caramiphen, ethylbenztropine) removed static tremor caused by D. Substances which inhibit intentional tremor of Wilson's type (penicillamine, alfa-methyl-dopa) (38) even increase this tremor. Alfa-methyl-meta-tyrozine, DL-4-chloramphetamine, gamma-hydroxybutyrate, L-Dopa, and 5-hydroxytryptophane, are without effect.

D is without effect on the activity of cholinesterase in erythrocites and brain of rat after 30, 60 and 180 mins.*

From table VI we see that the application of D leads to a reduction of temperature as well as to analgesia and an increase of the blood sugar.

Table VI

THE AMOUNT OF DOPAMINE ug/g IN FRESH TISSUE OF RAT BRAIN TREATED PREVIOUSLY BY D

Substance and dose	ug DOPAMINE / g tissue		
	Control	4h	24h
D 100 mg/kg i. p.	0,60 (±0,12—6)	0,60 (±0,12—6)	0,51 (0,11—12) $p<0,01$

From table VI we see that dopamine is reduced after 24 hours.

The injection of D directly into specific regions of the CNS led to expressed static tremor only when we gave 10 gamma and only in the nucleus niger.

* Our thank a due too Dr. B. Bošković (ITMZ, Beograd) who measured the activity of cholinesterase.

Table V

THE EFFECT OF α, α′ DIPYRIDYL (100 mg/kg i. p.) ON RAT BODY TEMPERATURE, PAIN AND BLOOD SUGAR OF THE RAT

a) Rat body temperature

Body temperature of control animals	Body temperature after the application of α, α′ dipyridyl					p
	2 hours	2.5 hours	3 hours	4 hours	24 hours	
1	2	3	4	5	6	7
37,86±0,28(6)	33,33±0,36(6)	32,83±1,01(6)	32,4±0,65(5)	32,68±0,52(5)	36,92±0,42(5)	1:2<0,01 1:3<0,01 1:4<0,01 1:5<0,01 1:6>0,05

b) Pain

Reaction to pain before the aplication of α, α'-dipyridyl	Reaction to pain 2 hours after the aplication of α, α'-dipyridyl	p
2,10±0,77(9)	5,16±0,75(9)	1:2 < 0,05

c) Blood sugar

Blood sugar concentration of control animals	Blood sugar concentration after the application of α, α'-dipyridyl			p
	2h	4h	24 h	
122±4,22(6)	158±10,61(6)	162±12,39(6)	120±10,17(5)	1:2<0,05 1:3<0,05 1:4>0,05

All numbers in the table represent the mean values + of standard error
The numbers in brackets represent the number of treated animals

DISCUSSION

As we see D causes static tremor surprisingly similar to tremor caused by OT in its biochemical effects. There is an increase in ACh in CNS as well as with OT (22, 30). This tremor can be inhibited with known anti-parkinsonic agents, although slightly higher doses are needed for this than in tremor caused by OT, except for ethyl-benztropine which is known to act entirely centrally (45). D as well as OT (22) does not inhibit cholinesterase which we think is very interesting, because it follows from this that the increase of ACh is produced because of some other reasons or because of the activation of cholineacetylase, which we have already found for OT (32). It is also interesting that D leads to a reduction in TFe, TFl and copper, which is known also for OT (39, 40, 20, 18). Tremor caused by D also reduced temperature and blood sugar, and caused analgesia, the same as OT. We see that substances which reduce or increase dopamine and serotonine in CNS are without effect on D tremor, and we see a similarity of action of D and OT. We see that D changes the quantity of dopamine in the brain of rat after 24 hours.

It is very interesting that injection of D only in the nucleus niger leads to tremor. We emphasise this especially because it is known that the region contains most TH (6). We have said that this enzyme is dependant on iron (46, 26) and it is known that D combines with iron. In connection with this it is interesting to mention that we showed earlier that tripyridine, which combines with iron too (29), also causes forced movements in mice similar to chorea (37). We showed earlier that deferoxamine, a substance which quite specifically combines with iron (45), if given intracranialy to mouse, also causes static tremor (45). Accordingly the question is, what is the cause of static tremor if we compare D and OT. We see that both substances lead to the same biochemical changes in CNS (increase of ACh, fall of TFe, TFl, and action of cholinesterase unchanged). On the other hand we showed that a continuous infusion of ACh in globus pallidus also causes static tremor (35).

We think that from all this it is possible to say that the reduction of iron is of primary importance in the formation of experimental tremor with either OT or D. With this it is necessary to emphasise that the globus pallidus and the nucleus niger are the richest regions in iron in the CNS (44, 32). The decrease in iron in those regions leads to an increase of ACh. We still do not know if it is because of the liberation of ACh from some depot or because of the activation of cholinacetylase. We also do not know the role of TFl.

In this observation it is significant that D as well as OT leads to a reduction of iron, that they do not inhibit cholinesterase and that they increase significantly ACh in CNS. However an infusion of ACh directly into the globus pallidus also causes static tremor (35). In accordance with all of these findings it looks as if an increase of ACh in CNS, which is the real cause of tremor,leads to a reduction of iron. That ACh is the direct provocator of static tremor is see after the application of chemicholine-3 directly into the globus pallidus which prevents static tremor caused by OT (34, 36).

We should emphasise that in patients with Parkinson's disease, there is an increase of elimination of iron in the urine if they are given an injection of deferoxamine (2).

We found that Cu^{++} is reduced after giving D as after OT (18). Cu is essential for the function of dopamine-beta-hydroxylase (11). Maybe because of this a reduction of dopamine is not found in acute experiments as on one hand TH increases its formation and the inactivation of dopamine-β-hydroxylase prevents its degradation.

Recently Bapta et al showed that D does not lead to inhibition of tyrosine-hydroxylase (1). According to them dopamine and serotonine do not change and the transformation of radioactive tyrosine in dopamine and tryptophan in serotonine develops normally if animals receive D (1). Our results are not quite the same because we found that dopamine was significantly reduced in the brain of rat, 24 hours after D was given. Besides this Bapta et al (1) could not remove rest tremor produced by D, with atropine, but we succeeded not only with atropine but with other antiparkinsonics too. On examining our findings, Taylor et al. (42), and those of Bapta et al. (1), it looks as if the reduction of iron is of essential importance, because we got the same results with an injection of deferoxamine in the globus pallidus (37). According to this, all that reduces iron in the CNS leads to static tremor which can be inhibited with antiparkinsonic substances. We did not measure the activity of tyrosine hydroxylase, and we cannot say if Taylor et al (42) or Bapta et al (1) is right. The fact is that the activity of tyrosine hydroxylase after D can be reactivated with the addition of Fe^{++} (42) According to our lastest experiments, an intracerebral injection of deferoxamine increased ACh in CNS too, which is also how oxotremorine and D work. Because of this it looks as if the reduction of iron leads to increased activity of cholineacetylase, which is already proved for tremorine (33) and recently als for OT (23). We cannot say mow if there is a reduction of tyrosine hydroxylase at the same time, but it seems that it is not essential as oxotremorine causes rest tremor but dopamine is certainly not reduced. It is difficult to say if the reduction of iron because of D, is important for the eventual inactivation of dopamine- beta-hydroxylase in vivo, because we found that copper decreases after D is given. It is already known that in vitro D reduces the activity of dopamine-beta-hydroxylase (12, 13).

Finally we would like to say that Voroshilovskaya and Reitses (48) found that adrenelectomy increased iron in CNS. We could indeed show that in adrenelectomised rats, tremor caused by oxotremorine and D either does not develop or is exceptionally weak in comparison with controls.

We should like to quote experiments with deoxyfrenolicine. This antibiotic inhibits TH in vitro and in vivo (43) However the inhibition cannot be removed with Fe^{++} in comparison to D. Direct injection of deoxyfrenolicine into the NN (10 gamma) did not cause tremor. We think that this also speaks for our hypothesis that a reduction of Fe in CNS is clear in certain regions, and is essential for the development of Parkinsonic tremor.

CONCLUSION

α-α-dipyridyl as well as oxotremorine, causes static tremor which can be removed by means of anticholinergics. This substance as well as oxotremorine usually causes biochemical changes, reduction of total iron and total flavines, and increases acetylcholine in the brain of rat. Further it reduces temperature, acts analgetically and increases the blood-sugar in the same way as oxotremorine. Taylor et al found that α-α-dipyridyl inhibits tyrosine hydroxylase while Bapta et al could not prove this.

On the basis of our own experiments it is concluded that for the existence of tremor caused by α-α-dipyridyl, oxotremorine or deferoxamine, the reduction of iron in the CNS is essential but the inhibition of tyrosine hydroxylase is not.

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ULOGA ŽELJEZA U IZAZIVANJU TREMORA

KRATAK SADRŽAJ

Budući da tirosin-hidroksilaza može vršiti svoju aktivnost samo u prisutnosti željeza, a ona je jedan od faktora u stvaranju dopamina, autori su ispitivali djelovanje alfa-alfa-dipiridila, jedne supstance koja veže na sebe željezo u odnosu na statički tremor. Pokazalo se je da alfa-alfa-dipiridil izaziva statički tremor istog tipa kao i oxotremorin, s istim biokemijskim promjenama i drugim manifestacijama koje su vezane uz djelovanje oxotremorina. Tako npr. alfa-alfa-dipiridil također snizuje ne samo željezo, nego i bakar i ukupne flavine u mozgu štakora. Ova supstanca dovodi i do pada temperature, zatim smanjuje osjet bola kao i oxotremorin. Budući da injekcija ove supstance u nucleus niger dovodi do statičkog tremora, a to isto uzrokuje i injekcija deferoxamina, zaključuje se da je željezo bitno za postanak tremora i da njegov pad uzrokuje porast acetilholina u mozgu. Sve ove vrste tremora imaju i tu zajedničku karakteristiku da se mogu inhibirati antiholinergicima.

REFERENCES

1. Bapta, J., Neff, N. H., Costa, E.: The mechanism of tissue norepinephrine depletion by α' α'-dipyridyl. Neuropharmacology **9**, 335—340 (1970).

2. Barbeau, A., Boileau, J. L.: Mobilizable iron in Parkinson's disease. Neurology **19**, 314 (1969).

3. Bessey, O. A., Lowry, O. H., Love, R. H.: The fluorometric measurement of the nucleotides of riboflavin and their concentration in tissues. J. Biol. Chem. **180**, 755—769 (1949).

4. Breljaković, A., Hadžović, S., Štern, P.: Ein billiger und präziser stereotaktischer Apparat für Ratten und Katzen. Z. med. Labortechnik **5**, 47—48, (1964).

5. Carlsson, A., Waldeck, B.: Acta Physiol. Scand. 44, 293—298 (1958). Citirano po Udenfriend, S. in »Fluorescence Assay in Biology and Medicine, Academic Press, New York, London, 1962, 137—140.

6. Cote, L. J., Fahn, S.: Properties of tyrosine hydroxylase in brain and its distribution in rhesus monkea brain. Fed. Proc. **27**, 752 (1968).

7. Foy, A. L., Williams, H. L., Cortell, S., Conrad, M. E.: A modified procedure for determination non-heme iron. Anal. Biochem. **18**, 559—562 (1967).

8. Friedman, A. H,- Everett, G. M.: Pharmacological aspects of Parkinsonism. Advances in Pharmacology **3**, Academic Press, New York, 83—127. (1964).

9. Fuller, R. W., Hines, C. W., Mills, J.: Lowering of brain serotonin level by chloramphetamines. Biochem. Pharmacol. **14**, 483—488 (1965).

10. Gessa, G. L., Crabai, F., Vargiu, L., Spano, P. F.: Selective increase of brain dopamine induced by γ-hydroxybutyrate: study of the mechanism of action. J. Neurochemistry **15**, 377 (1968).

11. Goldstein, M.: Dopamine-β-Hydroxylase: A copper enzyme. In »The biochemistry of Copper« ed. Peisach, J., Aisen, P., Blumberg, W. E., Academic Press, New York, 1966, 443—454.

12. Goldstein, M., Lauber, E., McKereghan, M. R.: The inhibition of dopamine-β-hydroxylase by tropolone and other chelating agents. Biochem. Pharmacol. **13**, 1103—1106 (1964).

13. Green, A. L.: The inhibition of dopamine-β-oxidase by chelating agents. Biochem. Biophys. Acta. **81**, 394—397 (1964).

14. de Groot, J.: The rat forebrain in stereotaxic coordinates. Verhandlingen der Koninklijke Nederlands, Akademie van Wetenschappen, AFD, Natuurkunde, Amsterdam, **52**, 3 (1955).

15. Gubler, C. J., Lahey, M. E., Henbrucker, H. A., Cartwright, G. E., Wintrobe, M. M.: Determination of copper in blood and plasma. J. Biol. Chem. **196**, 209 (1952). Cit. Hinsberg, K., Lang, K., Medizinischen Chemie, Urban u. Schwarzenberg, München 1957, 40.

16. Gupta, M., Ganguly, D. K.: Metabolic effects of oxotremorine. Arch. int. Pharmacodyn. **180**, 191—194 (1969).

17. Hadžović, S., Nikolin, B., Štern, P.: The effect of tremorine and lysergic acid diethylamide on the iron content of the rat brain. J. of Neurochemistry **12**, 908—909 (1965):

18. Hadžović, S., Košak, R., Štern, P.: The effect of tremorigenic substances on the copper content of the brain. J. Neurochem. **13**, 1027—1029 (1966).

19. Hadžović, S., Nikolin, B., Štern, P.: The iron content of the brain of rats with choreiform movements. Europ. J. of Pharmacol. **1**, 15—17 (1967).

20. Hasanagić, E., Štern, P.: Changes of total flavines in different parts of the rat brain under the influence of oxotremorine. Life Sciences **7**, 921—924 (1968).

21. Heilmeyer, L., Keiderling, W., Stuwe, G.: Kupfer und Eisen als körperelgene Wirkstoffe und ihre Bedeutung bei Krankheit geschehen. Fisher, Jena (1941).

22. Holmstedt, B., Lundgreen, G.: Tremorogenic agents and brain acetylcholine in »Mechanisms of release of biogenic amines«. Pergamon Press, Oxford, 439—468 (1966).

23. Holmstedt, B., Lundgreen, G.: Effects of oxotremorine and atropine on choline acetylase activity. In: Heilbronn, E., Winter, A, »Drugs and cholinergic mechanisms in the CNS« Research Institute of National Defence, Stockholm, 1970, 339—354.

24. Holmstedt, B., Lundgreen, G., Sundwall, A.: Tremorine and atropine effects on brain acetylcholine. Life Sciences **10,** 731—736 (1963).

25. Hornykiewicz, O.: Dopamine (3-Hydroxytyramine) and brain function. Pharmac. Rev. **18,** 925—964 (1966).

26. Levitt, M., Gibb, J. W., Daly, J. W., Lipton, M., Udenfriend, S.: A new class of tyrosine hydroxylase inhibitors and a simple assay of inhibition in vivo. Biochem. Pharmacol. **16,** 1313—1321 (1967).

27. McIntosh, F. C., Perry, W. M. L.: Biological estimation of acetylcholine. Ment. med. Res., **3,** 78—92 (1950).

28. Michelson, H. O.: An electrometric method for the determination of red blood cell and plasma cholinesterase activity. J. Lab. Chlin. Med. **34,** 1564—1568 (1949).

29. Moss, M. L., Mellon, G.: Colorimetric determination of iron with 2,2'-bipyridyl and with 2,2',2"-terpyridyl. Ind. Eng. Chem. **14,** 862 (1942).

30. Pepeu, G.: Effect of »Tremorine« and some anti-parkinson's disease drugs on acetylcholine in rat's brain. Nature. **200,** 895 (1963).

31. Popov, N., Pohle, W., Rösler, W., Matthies, H.: Regionale Verteilung von γ-Aminobuttersäure, Glutaminsäure, Asparaginsäure, Dopamin, Noradrenalin und Serotonin im Rattenhirn. Acta biol. med. german. **18,** 695—702 (1967).

32. Rafaelsen, O. J., Kofod, B.: Iron from »Handbook of neurochemistry (Lajtha, A. ed.)« **1,** Plenum Press, New York (1969) 261—271.

33. Ratković, D., Štern, P., Bošković, B.: Über die Wirkung des Tremorins auf die Cholinacetylase. Bull. Sci. Cons. Acad. R. P. F. Yugoslav. **10,** 40 (1965).

34. Ruždić, N.: Promjene tremorinskog efekta pod dejstvom intrapalidalno apliciranih supstanci. Magistarski rad, Sarajevo, 1965.

35. Ruždić, N., Štern, P.: The importance of globus pallidus in the production of rest tremor. Med. Pharmacol. exp. **14,** 17—23 (1966).

36. Slater, P., Rogers, K. J.: The effects of triethylcholine and hemicholinium-3 on tremor and brain acetylcholine. Europ. J. Pharmacol. **4,** 390—394 (1968).

37. Štern, P., Huković, S.: Pharmacological analysis of 2,2',2"-tripyridine. Folia Med. Facult. Med. Univ. Saraev. 59—72 (1966).

38. Štern, P., Kuljak, S.: Tremor induction in mice and its modification. Europ. J. Pharmacol. **5,** 343—347 (1969).

39. Štern, P., Hadžović, S., Nikolin, B.: Bedeutung des Eisens im Corpus striatum für den Tremor. Arch. Pharmak. exp. path. **257, 67** (1967).

40. Štern, P., Hasanagić, E., Fuks, Ž.: The influence of oxotremorine on iron and flavines in mitochondria from rat brain corpus striatum. Biochem. Pharmacol. **18**, 940—942 (1969).

41. Taeschler, M., Weidmann, H., Cerletti, A., Zur Pharmakologie von Ponalid, einen neuen zentralen Anticholinergicum. Schweiz. Med. Wschr. **92**, 1542—1545 (1962).

42. Taylor, R. J., Stubbs, C. S., Jr. Ellenbogen, L.: Tyrosine hydroxylase inhibition in vitro and in vivo by chelating agents. Biochemical. Pharmacology **18**, 587—594 (1969).

43. Taylor, R. J., Stubbs, C. S., Jr., Ellenbogen, L.: Tyrosine hydroxylase inhibition in vitro and in vivo by deoxyfrenolicin. Biochem. Pharmacol. **19**, 1737—1741 (1970).

44. Tingey, A. H.: The iron, copper and manganese content of the human brain. J. Ment. Sci. **83**, 452—460 (1937).

45. Tripod, J.: Tierexperimenteller Nachweis der Affinität von Desferal für Eisen, Schweiz. Med. Wschr. **93**, 969—973 (1963).

46. Udenfriend, S.: B. Tyrosine hydroxylase. Pharmacol. Rev. **18**, 43—51 (1966).

47. Udenfriend, S., Zaltzman-Nirenberg, P.: On the mechanism of the norepinephrine release produced by α-methyl-meta-tyrosine. J. Pharm. **138**, 194—199 (1962).

48. Vorošilovskaja, S. P., Raices, V. S.: Vlijanie udalenija nadpočečnih železna soderžanie medi, cinka i železa v golovnom mozgu životinjih. Patologičeskaja fiziologija i eksperimentalnaja terapija **10**, 78—80 (1966).