less skeletal deposition of these metals; and CATSCH and DU KHUONG LÊ[3] discovered certain chelating agents to be more effective than EDTA for removing cerium from the skeleton.

Table

20 h urinary excretion of aluminium from animals after injection in chelated form

Metal chelate[a]	Percentage[b] of chelate saturated with aluminium	Urinary excretion as percentage of dose	
		c	d
Al-diethylenetriamine pentaacetic acid	96	25	27
Al-ethylenediamine N-(2-hydroxycyclohexyl)-N,N', N'-triacetic acid	94	35	38
Al-ethylenediamine tetraacetic acid	98	40	41
Al-ethylenediamine-N-(2-hydroxyethyl)N,N',N'-triacetic acid	83	80	50
Al-ethylenediamine,-N,N'-bis-(2-hydroxycyclohexyl)-N, N'-diacetic acid	87	80	56
Al-1,2-diaminocyclohexane-N, N,N',N'-tetraacetic acid . . .	90	95	95
Al-polyphosphate	unknown	42	—

[a] Chelates (containing 1 mg Al) injected intraperitoneally into 450 g male guinea pigs, except Al-tripolyphosphate where 7 mg Al with 190 mg Na-polyphosphate was injected intravenously into a 1500 g female rabbit. Injection solutions were all at pH 7·4.

[b] The percentage saturation of the chelate may influence the fate of the metal (see experiments on yttrium[4]).

[c] Determined by the method of GENTRY and SHERRINGTON[5] on ashed aliquots; results of replicates quoted to the nearest 5%.

[d] Determined by the method used in [a] but on spots cut from paper chromatograms [6] (*Rf* about 0·07) after running aliquots; results are from single runs quoted to the nearest 1%.

Aluminium is now reported to be only partially excreted in the urine after injection into guinea pigs, rabbits and rats as Al-EDTA. Rabbits injected intravenously with large doses (18 mg Al/kg) died after a few days and showed mottling of the livers and to a lesser degree of the kidneys. The livers had undergone fatty change and necrosis in the periphery of the lobules and the kidneys showed tubular necrosis. A rat also died after eight days using the same dose per kg. Aluminium chelates were, however, well tolerated at reasonable dose levels; for example, a 300 g male rat injected intravenously with 3 mg of aluminium (as Al-EDTA) is apparently healthy eight months later, and a pregnant female guinea pig gave birth to a normal litter 52 days after intraperitoneal injection of 2 mg of aluminium as the 1,2-diaminocyclohexane N,N,N',N'-tetraacetic acid chelate. Urinary excretions of aluminium varied with the chelating agent used (Table).

Most of the urinary aluminium was in the sediments, presumably in the form of aluminium phosphate. Aqueous Al-EDTA (25 ml containing 75 mg Al, pH 7·4) was allowed to stand for 86 h at 37°C with powdered defatted ox shin bone (5 g); on treating the sediment with N HCl, 6 mg of aluminium were dissolved off. There was 15 mg of calcium in the supernatant. Al-EDTA reacted even with aqueous sodium orthophosphate at 25°C (pH 7) to give a small precipitate of $AlPO_4$. The reason for the skeletal deposition of Group III metals[2,3] may therefore lie in the stability of their phosphates. The question as to whether calcium chelates are excreted following administration of Group III metal chelates remains, but is not the object of this investigation.

We thank Mrs. O. L. KNIGHT and Mr. M. D. MIDDLETON for assistance with some experiments. Messrs. GEIGY of Basle presented the chelating agents and AMCOH, South Africa, the sodium tripolyphosphate. – This report is published by permission of the South African Council for Scientific and Industrial Research.

D. A. SUTTON and L. W. MARASAS

Pneumoconiosis Research Unit of the Council for Scientific and Industrial Research at the South African Institute for Medical Research, Johannesburg, July 13, 1959.

Zusammenfassung

Der Grad der Aluminiumausscheidung im Urin hängt, nach seiner Verabreichung in Form von Aluminiumchelat, vom verwendeten Chelat ab. Dies ist in Übereinstimmung mit anderen Metallen der Gruppe III. Die Ablagerung dieser Metalle im Skelett beruht wahrscheinlich auf der Stabilität ihrer Phosphate.

Increase of Free 5-Hydroxytryptamine in Blood Plasma by Reserpine and a Benzoquinolizine Derivative

Rauwolfia alkaloids and benzoquinolizine derivatives cause a decrease of the 5-hydroxytryptamine (5 HT) content in various tissues[1]. Thereby the excretion of 5-hydroxyindole acetic acid (5 HIAA), a major metabolic product of 5 HT, is increased in the urine[1]. In isolated blood platelets the 5 HT decreases also after addition of reserpine or the benzoquinolizine derivative 2-oxo-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-benzo[a]quinolizine as methan sulfonate (BQ)[3,4]. It has been suggested that Rauwolfia alkaloids and benzoquinolizine derivatives act by releasing the 5 HT from the binding site in the tissue. The released 5 HT would then be oxidized by monoamine oxidase (MAO) and excreted as 5 HIAA in the urine[5]. There is, however, no proof that 5 HT is liberated as such; it might be metabolized, e. g. at the binding sites, and released as 5 HIAA. Evidence for the first possibility consists in the observation that incubation of rabbit platelets with reserpine in an atmosphere of nitrogen causes an increase of the amount of 5 HT in the plasma[6].

[1] A. PLETSCHER, P. A. SHORE, and B. B. BRODIE, Science *122*, 374 (1955); J. Pharmacol. exper. Therap. *116*, 84 (1956). – P. A. SHORE, A. PLETSCHER, E. G. TOMICH, R. KUNTZMAN, and B. B. BRODIE, J. Pharmacol. exper. Therap. *117*, 232 (1956). – B. B. BRODIE, P. A. SHORE, and A. PLETSCHER, Science *123*, 992 (1956). – M. K. PAASONEN and M. VOGT, J. Physiol. (Lond.) *131*, 992 (1956). – A. PLETSCHER, H. BESENDORF, and H. P. BÄCHTOLD, Arch. exper. Path. Pharmak. *232*, 499 (1958).

[2] P. A. SHORE, S. L. SILVER, and B. B. BRODIE, Science *122*, 284 (1955).

[3] Trade name Nitoman.

[4] A. CARLSSON, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. exper. Therap. *120*, 334 (1957). – G. P. QUINN, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. exper. Therap. *127*, 103 (1959).

[5] B. B. BRODIE, A. PLETSCHER, and P. A. SHORE, Science *122*, 968 (1955).

[6] A. CARLSSON, P. A. SHORE, and B. B. BRODIE, J. Pharmacol. exper. Therap. *120*, 334 (1957).

[3] A. CATSCH and DU KHUONG LÊ, Nature *180*, 609 (1957).

[4] H. C. DUDLEY, J. Lab. clin. Med. *45*, 792 (1955).

[5] C. H. R. GENTRY and L. G. SHERRINGTON, Analyst *71*, 432 (1946).

[6] I. I. M. ELBEITH, J. F. W. MCOMIE, and F. H. POLLARD, Disc. Faraday Soc. *7*, 183 (1949).

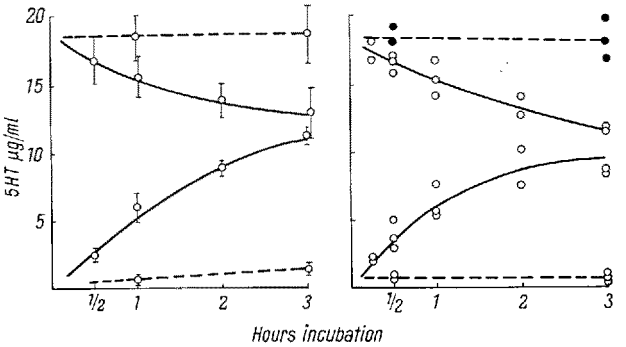
The decrease of the total 5HT in various tissues which is caused by reserpine and BQ can be counteracted by pretreatment of the animals with MAO inhibitors such as iproniazid[7,8]. It is not yet decided whether the breakdown of 5HT released by reserpine and BQ is diminished by MAO inhibitors or whether the latter drugs inhibit the liberation of 5HT induced by reserpine and BQ. Injection of the Rauwolfia alkaloid raunescine causes an increase of the 5HT in the plasma of rats and rabbits after iproniazid pretreatment[9]. This probably suggests that the metabolism of released 5HT is inhibited. The origin of this 'free' 5 HT remains, however, unknown.

The present work was undertaken to study whether reserpine and BQ liberate 5HT as such from isolated platelets and whether the amount of free 5HT in the plasma can be increased by a combined treatment with a MAO inhibitor and reserpine or BQ, respectively.

Methods. 15 h after i. p. injection of 20 mg/kg of 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl) hydrazine HCl (BMIH)[10] arterial blood was taken by means of a polyethylene cannula from heparinized rabbits in ether anesthesia, weighing 2·5 to 3·5 kg and fasted for 16 h. Untrated animals served as controls. The blood was immediately mixed with 1/9 volume of 1% disodium ethylenediamine tetraacetate in saline. After centrifuging at 200–300 × g for 5 min the platelet rich part of the plasma was withdrawn and the procedure repeated once or more. 3–4 ml of plasma were incubated with reserpine, BQ or the solvents alone under gentle shaking for 30–180 min in 25 ml Erlenmeyer flasks at 37°C in room atmosphere. After centrifugation at about 1700 × g for 25 min at room temperature the 5HT was measured by a spectrophotofluorometric method in the platelet poor plasma (supernatant) and in the platelets (sediment[12]). Reserpine was dissolved in glacial acetic acid, diluted with water to a final acetic acid concentration of not more than 0·02% and 0·025 ml of this solution added into each ml of plasma. Final concentration of reserpine: 1 μg/ml 0·05 ml. BQ was dissolved in saline and 0·05 ml of this solution was added per ml of plasma to yield a final concentration of 20 μg base/ml plasma. All the experiments were carried out in siliconized glassware.

Platelet counts were made according to FEISSLY[13] in the phase contrast microscope. No decrease could be found in the number of platelets during incubation with reserpine or BQ indicating that the platelets were not destroyed.

Results. — (1) In the platelets of rabbits pretreated with BMIH, incubation with reserpine as well as with BQ caused a progressive decrease of the 5HT. The 5HT in the plasma increased continuously, amounting to about 45% of the total amine after 3 h. Incubation of the platelet rich plasma without any drug caused no appreciable change of the 5HT content in either platelets or plasma. The total amount of 5HT extracted from platelets and plasma after incubation with reserpine or BQ was somewhat higher than could be expected. The reason for this finding is not yet clear. It could possibly be due to a methodical error because of different recovery rates of the amine from platelets and plasma. This has, however, no importance for the interpretation of the results (Figure).



Amounts of 5-hydroxytryptamine in platelets and platelet-free plasma after incubation *in vitro* with 1 μg/ml reserpine (a) or 20 μg/ml benzoquinolizine derivative (b). The animals were pretreated with a monoamine oxidase inhibitor of the hydrazine type (BMIH) (20 mg/kg i.p. 15 h before bleeding). The curves from above down indicate:

no reserpine incubation, platelets;
reserpine incubation, platelets;
reserpine incubation, plasma;
no reserpine incubation, plasma.

Each value represents the mean of four experiments.
Vertical lines: standard error.
The 5HT values indicated correspond to the amount of platelets and plasma, respectively, contained in 1 cm³ of platelet-rich plasma.

(2) In the platelets of controls, incubation with 1 μg/ml reserpine caused a decrease of the 5HT content to 77·0 ± 5·0% of the preincubation values after 1 h ($p > 0{\cdot}05$) and to 56·6 ± 5·4% after 3 h ($p < 0{\cdot}01$). In the plasma there was no significant increase of the 5HT concentration compared with controls which were incubated without reserpine ($p > 0{\cdot}05$) (controls: 1 h: 3·2 ± 1·5%; 3 h: 3·5 ± 1·4%. Reserpine incubation: 1 h: 5·6 ± 1·5%; 3 h: 5·5 ± 2·6%).

Discussion. — The results show that in platelet rich plasma of rabbits pretreated with the MAO inhibitor BMIH the free 5HT in the plasma increases markedly after incubation with reserpine and BQ. The 5HT rise found in the plasma corresponds to the 5HT decrease in the platelets. This is strong evidence for the fact that reserpine as well as BQ are able to liberate 5HT as such from the platelets. The findings demonstrate furthermore that reserpine and BQ still cause liberation of 5HT if MAO is inhibited. This supports the assumption of increased free 5HT in the tissues after combined treatment with MAO inhibitors and Rauwolfia alkaloids or benzoquinolizine derivatives respectively. The question as to whether MAO inhibitors also inhibit the reserpine induced liberation of 5HT from platelets has been investigated separately[14].

After incubation of platelet rich plasma from non-pretreated animals with reserpine, the 5HT in the platelets decreases without significant increase of 5HT in

[7] Trade name Marsilid.

[8] B. B. BRODIE, A. PLETSCHER, and P. A. SHORE, J. Pharmacol. exper. Therap. *116*, 9 (1956). – A. PLETSCHER, Exper. *12*, 479 (1956). – P. A. SHORE and B. B. BRODIE, Proc. Soc. exper. Biol. Med., N. Y. *99*, 433 (1957).

[9] N. T. KÄRKI and M. K. PAASONEN, The Second Scandinavian Summer Meeting of Biochemists, Pharmacologists, and Physiologists, joint Meeting with the British Biochemical Society in Turku (Finland), August 27–29, 1959.

[10] MAO inhibitor with long duration of action, similar to iproniazid but 10–20 times more potent[11]. Trade name: Marplan.

[11] L. O. RANDALL and R. E. BAGDON, Ann. N. Y. Acad. Sci. *80*, 626 (1959).

[12] S. UDENFRIEND, H. WEISSBACH, and C. T. CLARK, J. biol. Chem. *215*, 337 (1955).

[13] R. FEISSLY, Schweiz. med. Wschr. *84*, 808 (1954).

[14] M. K. PAASONEN and A. PLETSCHER, Exper., in press (1960).

plasma. This and the previous results indicate that blood is able to destroy endogenous 5HT through an enzyme system which can be inhibited by MAO inhibitors. This finding confirms earlier experiments which showed that human blood serum oxidizes added 5HT, an effect which is inhibited by the MAO inhibitor iproniazid[15]. The increase of 5HT in the plasma after incubation of platelets with reserpine in N_2 atmosphere[6] can probably also be attributed to inhibition of this enzyme. It is not known, however, whether the breakdown of the 5HT derived from platelets takes place in the platelets or in the plasma.

M. K. Paasonen* and A. Pletscher

Medizinische Abteilung der F. Hoffmann-La Roche & Co. & Co. A.G., Basel, October 5, 1959.

Zusammenfassung

Nach Vorbehandlung von Kaninchen mit einem Monoaminoxydase-Hemmer des Hydrazin-Typs wurde plättchenreiches Plasma mit Reserpin und einem Benzochinolizin-Derivat inkubiert. Im Plasma kam es dadurch zu einem Anstieg des nicht an Thrombozyten gebundenen 5HT, der dem Abfall des Amins in den Thrombozyten entsprach. Bei Kontrolltieren (ohne Monoaminoxydase-Hemmer) führte die Inkubation von plättchenreichem Plasma mit Reserpin zu keinem Anstieg des freien 5HT im Plasma, obgleich es in den Thrombozyten zu einer starken Senkung des Amins kam.

[15] G. Martin, N. Eriksen, and E. P. Benditt, Fed. Proc. *17*, 447 (1958).

* Guest worker from the Department of Pharmacology, University of Helsinki (Finland). Supported by a grant from the Finnish Medical Society *Duodecim*.

Über die villikininartige Wirkung des Harnes

Die Darmzottenbewegung wird hormonal durch das Villikinin gesteuert, das durch sekretogene Impulse aus der Dünndarmschleimhaut freigesetzt wird[1,2]. Die Wirkung des Villikinins verschwindet nach Ganglienblokkade[3].

Es wurde untersucht, ob die intravenöse Verabreichung von Menschenharn die Darmzottenbewegung beeinflusst. Die Versuche wurden an 23 Hunden nach der Methode von Kokas und Ludány durchgeführt[4]. Den Tieren wurde frischer nativer Harn von Gesunden bei nüchternem Magen intravenös verabreicht. Ferner wurde der Harn mit Norit ausgeschüttelt, zum Siedepunkt erhitzt und nach Abkühlen filtriert. Der derart aufgearbeitete Harn wurde ebenfalls intravenös verabreicht.

Sowohl der native Harn als auch der mit Norit ausgeschüttelte und zum Siedepunkt erhitzte Harn riefen ungefähr gleich starke Darmzottenbewegungen hervor. (Abbildung.) Diese dauert nach der niedrigsten Dosis 10 min, nach der höchsten Dosis länger als 30 min. Der native Harn bewirkte Blutdrucksenkung, der mit Norit ausgeschüttelte und zum Siedepunkt erhitzte Urin war ohne Einfluss auf den Blutdruck. In seiner Wirkung ähnelt der mit Norit ausgeschüttelte und zum Siedepunkt erhitzte Harn dem Villikinin, das ebenfalls nicht an Norit absorbiert wird, thermostabil ist und den Blutdruck nicht beeinflusst.

Es ist unwahrscheinlich, dass die Darmzottenbewegung nach intravenöser Applikation des Harnes durch H-Substanzen oder die Substanz P[5] verursacht wird, da diese beiden Stoffe eine Blutdrucksenkung hervorrufen, der mit Norit ausgeschüttelte Harn jedoch den Blutdruck nicht verändert.

Ich danke Herrn Prof. Ludány und Herrn Dr. Gáti aus dem Pathophysiologischen Institut in Budapest für die Überprüfung und Bestätigung meiner Ergebnisse. Diese Forscher haben mich darauf aufmerksam gemacht, dass die Darmzottenbewegung, welche durch Villikinin sowie auch durch intravenöse Gaben von Harn ausgelöst wird, durch Hexamethonium blockiert werden kann.

A. Svatoš

Forschungsinstitut für Pharmazie und Biochemie, Prag (Tschechoslowakei), 7. Juli 1959.

Summary

Intravenous application of human urine to dogs has similar effects as villikinin. Noriteabsorbed, heated and filtered urine stimulates the movement of the inestinal villi but has no effect on blood pressure.

[1] E. Kokas und G. Ludány, Pflügers Arch. *232*, 293 (1933).

[2] E. Kokas und G. Ludány, Pflügers Arch. *234*, 182 (1934).

[3] T. Gáti, G. Ludány und A. Sántha, Arch. int. Pharmacodyn. *113*, 390 (1958).

[4] E. Kokas und G. Ludány, Pflügers Arch. *231*, 20 (1932).

[5] G. Ludány, T. Gáti, St. Szabo und J. Hideg, Arch. int. Pharmacodyn. *118*, 62 (1959).

Changes of Evoked Potentials in Lateral Geniculate Body and Visual Cortex during Repetitive Photic Stimulation in the 'Cerveau isolé' Cat[1]

Following prolonged repetitive photic stimulation, the evoked potentials recorded from the visual cortex and the lateral geniculate body decrease markedly in amplitude. This phenomenon, usually referred to as 'habituation'[2,3] has been attributed to the activity of the ascending reticular system. In an attempt to ascertain the role of the reticular formation in the process of visual habituation, we have performed experiments which are reported below.

'Cerveau isolé' cats with intercollicular transection were used. The electrocortical activity was recorded monopolarly on an inkwriter, while a CRO was used for recording the evoked potentials of the primary visual cortex and of the lateral geniculate body. The photic stimulation consisted of 1/s-flashes of white light, the intensity of which was held constant throughout the period of the experiment. Olfactory stimulation was used for producing 'dishabituation'[2,3]. The mesencephalic reticular formation was stimulated through concentric elec-

[1] This research has been sponsored jointly by the Office of Scientific Research of the Air Research and Development Command, United States Air Force, through its European Office, under Contract No. AF 61(052)-107 and by the Rockefeller Foundation.

[2] R. Hernández-Peón, C. Guzman-Flores, M. Alcaraz, and A. Fernandez-Guardiola, Fed. proc. *15*, 91 (1956).

[3] R. Hernández-Peón, C. Guzman-Flores, M. Alcaraz, and A. Fernandez-Guardiola, Acta neurol. latinoamer. *4*, 121 (1958).