tion state of the retina is studied in detail on the ERG of the isolated frog's retina using statistical methods[7]. For the mammalian retina the reduction of the dark adapted *b*-wave caused by strychnine-concentrations $7\times10^{-5}$–$7\times10^{-4}M/l$ is significant with $p<0.025$ ($n=6$, $x_n-y_n \geqq 19\%$ and $\leqq 95\%$) tested by the sign test. The transitory increase of the *b*-wave elicited by strychnine-concentrations $3\times10^{-6}$–$3.5\times10^{-5}M/l$ is significant with $p<0.025$ ($n=6$, $x_n-y_n \geqq 10\%$ and $\leqq 107\%$). In the last case the retina was slightly light adapted by repeating the stimulus every 10 or 20 sec.

The alteration of the ERGs of frog's and mammalian retina by strychnine means that strychnine already acts on the second neuron because most components of the ERG originate there[8,9]. From this the conclusion may be drawn that the effect is caused by alteration of an inhibition mechanism in the layer of the second neuron. The lack of influence of strychnine on the ERG in the experiments of WOHLZOGEN and DANIS[3,4] may perhaps be explained by the barbiturate anaesthesia of the animals in their experiments. In the clinic of strychnine intoxication, a barbiturate antagonism is known.

*Zusammenfassung.* Strychnin in einer Konzentration von $3\times10^{-6}$ bis $3{,}5\times10^{-5}M/l$ verursacht eine vorübergehende Vergrösserung der *b*-Welle des ERG der isolierten Kaninchennetzhaut, in einer Konzentration von $7\times10^{-5}$ bis $7\times10^{-4}M/l$ verkleinert es die *b*-Welle. Aus den Ergebnissen wird geschlossen, dass die Substanz zumindest teilweise schon auf die Schicht des zweiten Neurons wirkt.

W. VÖRKEL and R. HANITZSCH

*Carl-Ludwig-Institut für Physiologie, Liebigstrasse 27, 701 Leipzig (DDR), 14 August 1970.*

1 P. O. THERMAN, Acta Soc. Scie. fenn. *2*, 1 (1938).
2 T. TOMITA and A. FUNAISHI, J. Neurophysiol. *15*, 75 (1952).
3 F. X. WOHLZOGEN, Z. Biol. *108*, 217 (1956).
4 P. DANIS, *Contribution à l'étude électrophysiolique de la rétine* (Imprim. Méd. et Scie., Bruxelles 1959).
5 W. O. HEISS and H. BORNSCHEIN, Pflügers Arch. ges. Physiol. *286*, 1 (1965).
6 R. HANITZSCH and H. BORNSCHEIN, Experientia *21*, 484 (1965).
7 W. VÖRKEL and R. HANITZSCH, Acta biol. med. germ., im Druck.
8 T. TOMITA, J. opt. Soc. Am. *53*, 49 (1963).
9 R. HANITZSCH and J. TRIFONOW, Vis. Res. *8*, 1445 (1968).

## Autoradiographic Demonstration of Uptake and Accumulation of $^3$H-6-Hydroxydopamine in Adrenergic Nerves

6-Hydroxydopamine (6-OH-DA) causes a long-lasting depletion of the noradrenaline (NA) content in various sympathetically innervated organs and a functional failure of transmission at the sympathetic nerve endings[1–4]. The reason for this has been shown to be due to 6-OH-DA inducing a selective degeneration of the adrenergic nerves[5–8]. Therefore this compound has attracted greatest interest above all as a denervation tool both in the central[9,10] and peripheral nervous system[8,11,12].

The mechanism by which 6-OH-DA produces its effects is not quite clear, but denervation and pharmacological experiments have disclosed that 6-OH-DA in all probability has to be taken up and accumulated in the adrenergic nerves[7,13]. In order to prove this in a more direct manner the autoradiographic localization of $^3$H-6-OH-DA has been investigated in mouse iris after incubation of the tissue in vitro in a physiological medium containing $^3$H-6-OH-DA.

*Methods.* Albino mice (N.M.R.I., 25–30 g) were used in the experiments. The mice were sacrificed under light ether anaesthesia and the irides were dissected out and transferred to incubation flasks containing Krebs-Ringer bicarbonate buffer pH 7.4 (2 ml buffer per 2 irides) with and without $^3$H-6-OH-DA·HBr (12.4 mCi/mmole). Some irides used were sympathetically denervated by removal of the superior cervical ganglion 48 h before the experiment or by injection of $2\times50$ mg/kg 6-OH-DA·HCl i.v. (2 h, 16 h interval). The incubations were performed at +37 °C using a metabolic shaker.

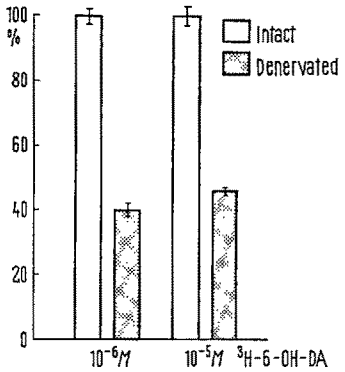



Fig. 1. In vitro uptake of $^3$H-6-OH-DA in intact and denervated mouse irides. Each column represents the mean ± S.E.M. of 6 determinations and is expressed as percent of the radioactivity taken up and accumulated in the intact organ. Uptake in intact irides (cpm/iris): $10^{-6}M$ 141 ± 3, $10^{-5}M$ 565 ± 15.

1 C. C. PORTER, J. A. TOTARO and C. A. STONE, J. Pharmac. exp. Ther. *140*, 308 (1963).
2 C. A. STONE, J. M. STAVORSKI, C. T. LUDDEN, H. C. WENGER, C. A. ROSS, J. A. TOTARO and C. C. PORTER, J. Pharmac. exp. Ther. *142*, 147 (1963).
3 R. LAVERTY, D. F. SHARMAN and M. VOGT, Br. J. Pharmac. *24*, 549 (1965).
4 C. C. PORTER, J. A. TOTARO and A. BURCIN, J. Pharmac. exp. Ther. *150*, 17 (1965).
5 J. P. TRANZER and H. THOENEN, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *257*, 343 (1967).
6 J. P. TRANZER and H. THOENEN, Experientia *24*, 155 (1968).
7 T. MALMFORS and CH. SACHS, Europ. J. Pharmac. *3*, 89 (1968).
8 H. THOENEN and J. P. TRANZER, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *261*, 271 (1968).
9 U. UNGERSTEDT, Europ. J. Pharmac. *5*, 107 (1968).
10 N. J. URETSKY and L. L. IVERSEN, Nature *221*, 557 (1969).
11 G. HAEUSLER, W. HAEFELY and H. THOENEN, J. Pharmac. exp. Ther. *170*, 50 (1969).
12 CH. SACHS, Acta physiol. scand. Suppl. 341 (1970).
13 G. JONSSON and CH. SACHS, Europ. J. Pharmac. *9*, 141 (1970).

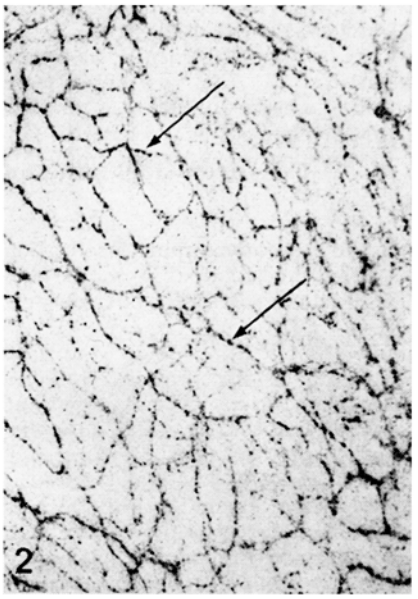
After termination of the incubation, the irides were re-incubated in fresh buffer for 10 min at +37 °C and thereafter prepared as whole mounts, dried and exposed to formaldehyde gas of optimum humidity for the histochemical demonstration of NA according to the method of FALCK and HILLARP[14,15].

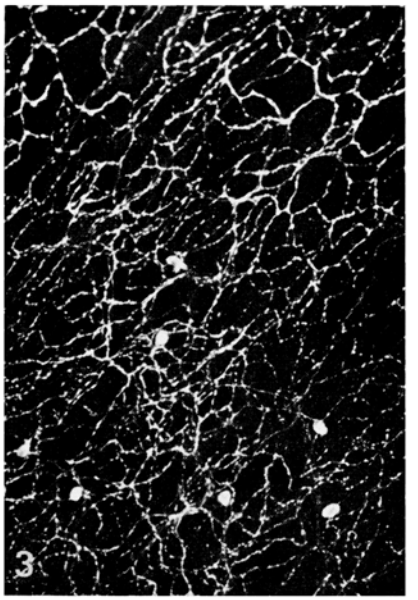
The irides were examined in a fluorescence microscope, photographed and covered with Ilford L4 emulsion (Ilford, Ltd., Essex, England) with the help of a loop technique[16,17]. After exposure for 2–8 weeks the irides were developed (Kodak D 19 B), fixed (Kodak X-ray Fixer and Replenisher) and examined in a Leitz Orthoplan light microscope.

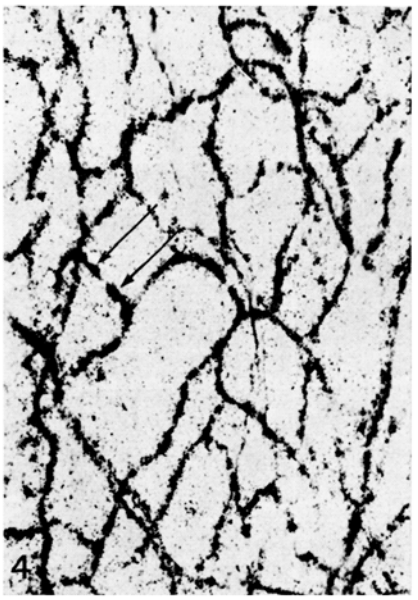
In one series of experiments the irides were, after the reincubation in fresh buffer, dissolved in 0.5 ml Soluene™-100 (Packard Instr. Co., Inc.). 10 ml toluene scintillation solution was added and radioactivity determined in a Packard Model 3320 Tri-Carb Liquid Scintillation Spectrometer.

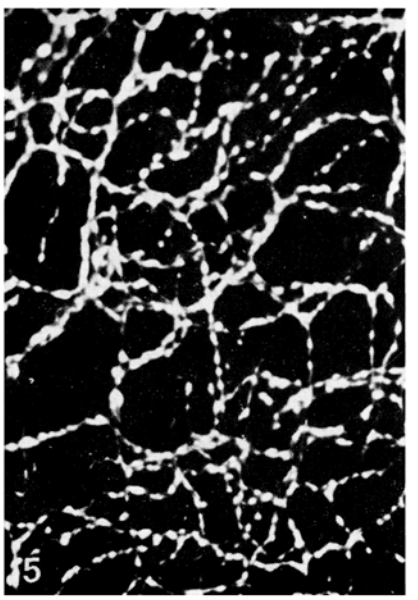
*Results.* Sympathetic denervation strongly reduces the uptake and accumulation of radioactivity in irides incubated for 60 min in a medium containing $10^{-6}M$ or $10^{-5}M$ $^3$H-6-OH-DA (Figure 1). The reduction of uptake is somewhat more pronounced when using the lower medium concentration of $^3$H-6-OH-DA. This is in accordance with results obtained from mouse atria[13]. The incubation in $10^{-6}M$ $^3$H-6-OH-DA did not cause any noticeable changes in the fluorescence morphological appearance of the adrenergic nerves, while $10^{-5}M$ resulted in a patchy disappearance of the fluorescent nerves. In a previous communication[13] it was shown that incubation in $5\times10^{-5}M$ or $10^{-4}M$ 6-OH-DA for 30 min gave a complete disappearance of the fluorescent

[14] T. MALMFORS, Acta physiol. scand. *64*, Suppl. 248 (1965).
[15] H. CORRODI and G. JONSSON, J. Histochem. Cytochem. *15*, 65 (1967).
[16] O. L. MILLER JR., G. E. STONE and D. M. PRESCOTT, J. Cell Biol. *23*, 654 (1964).
[17] T. NAGATA and T. NAWA, Histochemie 7, 370 (1966).




Figs. 2 and 4. Autoradiographs of stretch preparation of mouse iris, incubated with $^3$H-6-OH-DA ($10^{-5}M$, 60 min). Grains are concentrated in fiber-like strands often with a beaded appearance (arrows). A comparatively low activity is found between these strands. Magnifications: ×160 and ×500 respectively.

Figs. 3 and 5. Fluorescence micrographs of stretch preparation of mouse iris. The adrenergic nerve terminals form a dense groundplexus often consisting of single axons (cf. rat iris[14]). The NA is localized in especially high amounts in regularly spaced axonal enlargements, the so-called varicosities. Note the close similarity between the NA containing nerves (as seen in fluorescence micrographs) and the localization of $^3$H-6-OH-DA (as seen in autoradiographs). Magnifications: ×160 and ×500 respectively.

adrenergic nerves. 6-OH-DA itself does not give rise to any formaldehyde-induced fluorescence[13].

The autoradiographs (Figures 2 and 4) showed, after incubation of intact irides in $10^{-5}M$ $^3$H-6-OH-DA, an accumulation of grains in a pattern very closely resembling the morphology and distribution of the adrenergic nerves as demonstrated histochemically (Figures 3 and 5). Denervated irides did not disclose this accumulation but just a diffuse distribution of grains in a number similar to that observed between the accumulations of grains in the intact irides. It was not possible to demonstrate any accumulations of grains after incubation in $10^{-6}M$ $^3$H-6-OH-DA probably due to the low specific activity of the $^3$H-6-OH-DA used. Occasionally, accumulations of grains were observed in structures possibly representing mast cells.

*Discussion.* The results presented show that 6-OH-DA can be taken up and accumulated in adrenergic nerves. The evidence comes from the observations that after incubation in $^3$H-6-OH-DA the accumulation of grains have a distribution practically identical with that of the adrenergic nerves as revealed by fluorescence histochemistry. Furthermore, this accumulation can be prevented by sympathetic denervation. The grains observed spread diffusely in between the network represents the extraneuronal uptake of 6-OH-DA. At least part of the extraneuronal uptake might, however, also be localized in mast cells. 6-OH-DA in all probability uses the very efficient axonal 'membrane pump' for its inward transport and accumulation in the adrenergic neuron, since blockade of this uptake-accumulation mechanism either by desipramine or by incubation the tissue at 0 °C strongly reduces 6-OH-DA uptake[13] and prevents degeneration[7,13,18]. This property of 6-OH-DA certainly explains the fact that it produces a selective destruction of the adrenergic nerves[6,8]. Although it has been shown that 6-OH-DA can be taken up in the amine-storage granules intraneuronally, this does not seem to be a prerequisite for inducing degeneration[13,18,19].

*Zusammenfassung.* Die Regenbogenhaut der Maus wurde in einer physiologischen Pufferlösung mit radioaktivem 6-Hydroxydopamin ($^3$H-6-OH-DA) inkubiert. Mit Hilfe autoradiographischer und fluoreszenz-histochemischer Untersuchungen konnte gezeigt werden, dass $^3$H-6-OH-DA in die adrenergischen Nervenfasern der Regenbogenhaut aufgenommen und gespeichert wird. Dieser Befund bringt die direkte Erklärung der durch 6-Hydroxydopamin induzierten Degeneration der adrenergen Fasern.

Å. Ljungdahl, T. Hökfelt, G. Jonsson and Ch. Sachs[20]

*Department of Histology, Karolinska Intitutet, S-10401 Stockholm (Sweden), 28 August 1970.*

[18] T. Bennett, G. Burnstock, J. L. S. Cobb and T. Malmfors, Br. J. Pharmac. *38*, 802 (1970).

[19] J. B. Furness, G. R. Campbell, S. M. Gillard, T. Malmfors, J. L. S. Cobb and G. Burnstock, J. Pharmac. exp. Ther. *174*, 111 (1970).

[20] Acknowledgments. The authors are greatly indebted to F. Hoffmann-La Roche and Co., Basel, for supply of $^3$H-6-OH-DA·HBr and to Dr. H. Corrodi, AB Hässle, Göteborg, for 6-OH-DA·HCl. The investigation was supported by research grants from the Swedish Medical Research Council No. B71-14X-2887-02A and No. B71-14X-2295-04, Ollie and Elof Ericssons Stiftelse and Magnus Bergvalls Stiftelse.

## Influence of Coronary Dilators on the Uptake of Serotonin by Human Platelets and Adrenaline by Chromaffine Granules

Many drugs with different chemical structures have been used in the treatment of angina pectoris. Whereas their therapeutic value appears to be limited[1], there is no doubt about the ability of some of these compounds to interfere with adrenergic mechanisms[2], especially with the transport of catecholamines through subcellular and cellular membranes[3-6]. Since the membrane of human blood platelets shares certain properties with the axoplasma membrane of the adrenergic neuron, e.g., the active transport of biogenic amines[7], this model was chosen to investigate the effect of the 4 coronary dilators (see formula) prenylamine (Segontin®), oxyfedrine (Ildamen®), verapamil (Isoptin®) and carbochromenum (Intensain®) on the uptake of serotonin by human platelets. For comparison, the influence of these compounds on the uptake of adrenaline by chromaffine granules was examined.

*Materials and methods.* 1. Uptake of $^3$H-serotonin by human platelets. Blood was obtained from healthy donors by venous puncture with a plastic-syringe containing 1 ml 3.8% sodium citrate per 10 ml blood. Platelet-rich plasma was prepared as described by Zieve et al.[8]. Samples of 0.2 ml platelet-rich plasma containing about $4–6 \times 10^7$ platelets were incubated at 37 °C with 0.1 ml (1 μC) of $^3$H-serotonin (8.6 C/m$M$; Radiochem. Ceutre, Amersham) and 0.7 ml Krebs-Ringer-bicarbonate buffer (without $Ca^{++}$ and $Mg^{++}$) pH 7.4. The drugs were dissolved in buffer. Control samples were prepared by addition of $^3$H-serotonin to the platelets at 4 °C immediately before centrifugation. After incubation, the platelets were sedimented at $1200 \times g$ for 20 min and supernatants decanted. The sediments were lysed in 1 ml 0.01 $n$ HCl at 37 °C with shaking. Radioactivity was determined as previously described[3].

2. Uptake of $^{14}$C-adrenaline by chromaffine granules. Granules from bovine adrenal medulla were prepared

[1] M. Kaltenbach, H. J. Becker, V. Graef and H. Hunscha, Med. Klinik *65*, 494 (1970).

[2] D. Palm, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *263*, 159 (1969).

[3] H. Grobecker, D. Palm and P. Holtz, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *260*, 379 (1968).

[4] H. Grobecker and T. Malmfors, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *261*, 59 (1968).

[5] H. Grobecker, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path., in press (1971).

[6] D. Palm, H. Grobecker, I. J. Bak, Int. Symp. on New Aspects of Storage and Release of Catecholamines (Springer-Verlag, Bayer-Symposium 1970), vol. 2, p. 188.

[7] W. B. Abrams and H. M. Solomon, Clin. Pharmac. Ther. *10*, 702 (1969).

[8] P. D. Zieve, H. M. Solomon and J. R. Krevans, J. Cell. Physiol. *67*, 271 (1966).