behavioural syndrome produced parallels the strong central effects previously reported by CERLETTI.

The work presented here supports the concept that LSD acts, at least partly, through catecholamine mediation.

Zusammenfassung. Der Einfluss verschiedener Pharmaka auf das durch LSD hervorgerufene bizarre Verhalten der Ratte wurde untersucht. Die Resultate zeigen, dass α- und β-Blocker sowie α-Methyltyrosine eine starke Hemmung dieses Verhaltens erzeugen, im Gegensatz zu den Serotonin-Antagonisten Deseril, BOL-148 und Parachlorphenylalaninen, die nur schwache oder keine Hemmung hervorrufen. Es wird gefolgert, dass das bizarre Verhalten durch Wirkung von LSD an Katecholaminstrukturen hervorgerufen wird. Die Wirkung von 4 psychotropen Pharmaka am bizarren Verhalten wird im Rahmen dieser Hypothese diskutiert.

A. K. DIXON

Institute of Medical and Biological Research, Sandoz Ltd., Basel (Switzerland),
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PRO EXPERIMENTIS

Serendipitous Precise and Unique Staining of Cell Nuclei

We have recently been interested in developing new techniques and substrates for demonstration of enzymes in tissues, peripheral blood, bone marrow cells and bacteria. Previously we have described the application of the indigogenic principle to the histochemical demonstration of leucine aminopeptidase[1], B-glucosidase[2], B-galactosidase[3], *N*-acetyl-B-glucosaminidase[4], alkaline and acid phosphatase[5], sulfatase[6], B-xylosidase[7], endo and exo nucleases (phosphodiesterases[8]) serum alkaline and acid phosphatase by disc electrophoresis[9,10] and bacterial DNase[11]. The indolyl substrates were synthesized according to the methods described recently by HORWITZ and co-authors[12,13]. The indolyl substrates offer the advantage of precise enzyme localization with no or very little diffusion. Moreover, the substrates offer a simple and direct method for demonstration of the enzymes without the need for a coupling reaction. The principal of the indigogenic reaction is that a hydrolysis of the specific indolyl substrate occurs in the presence of the enzyme yielding a chromogenic highly insoluble indigo at the enzyme site. The addition of the redox system potassium ferro-ferricyanide is frequently included in the incubation medium to effect and accelerate the oxidation of the intermediate indoxyl to indigo[14].

In our recent studies on the intracellular localization of exonuclear and endonuclear 'phosphodiesterase' activity[8] one of the controls which were employed was an incubation of fresh frozen sections of various tissues in potassium ferro-ferricyanide at pH 5.2. We were surprised to observe selective and unique staining of only the cell nuclei of various tissues. We thus made this observation by serendipity.

Methods. Tissues from mouse and rat were used for this study. Representative pieces of tissue from each organ were removed and cut into blocks 2–4 mm in thickness, and quick-frozen by placing the tissue in a glass tube and immersing it in a Dewar flask containing acetone and dry ice at − 70 °C. The tissues were embedded in optimal cutting temperature compound, purchased from Lab-Tek, composed of water-soluble glycols and resins matched to a specific cutting zone temperature of − 20 °C to − 35 °C. The embedded tissue was then placed on the quick-freeze bar of a Lab-Tek cryostat for 1 min until the embedding medium was frozen, and became the proper consistency for cutting 6 μ sections at − 20 °C. After cutting, the sections were attached to warm slides. All solutions were maintained at 4 °C for preservation of enzymatic activity. The slides were then air dried to prevent the formation of ice crystals and stored at − 25 °C until incubated in the specific solution. Fresh frozen sections were incubated for 6 h in solutions consisting of 1 ml 0.05 *M* potassium ferrocyanide, 1 ml 0.05 *M* potassium ferricyanide, 10 ml 0.2 *M* acetate buffer pH 5.2. The reaction was observed hourly until 20 h. After incubation the slides were washed briefly in tap water and mounted in glycerol gel for microscopic examination.

Results. Selective and unique blue staining of only the cell nuclei of mouse and rat kidney, liver, skeletal muscle, spleen, gastrointestinal mucosa, gastrointestinal smooth muscle, pancreas and epididymis. The chromatin of the

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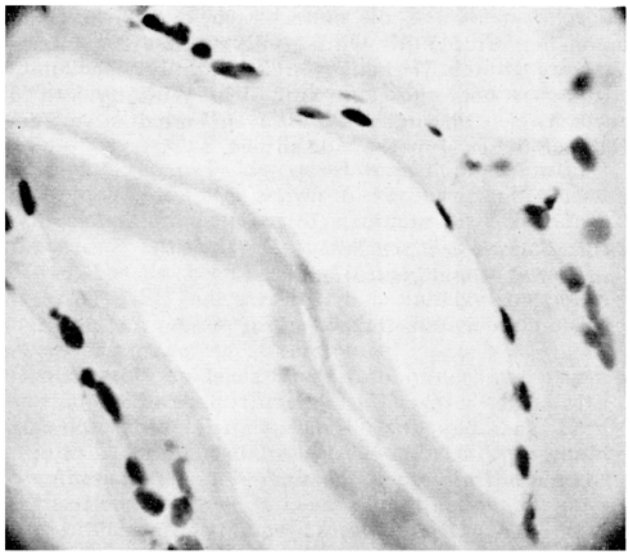

Fig. 1. Section of rat, skeletal muscle incubated in potassium ferro-ferricyanide at pH 5.2 demonstrating staining of nuclei of skeletal muscle cells. × 135.

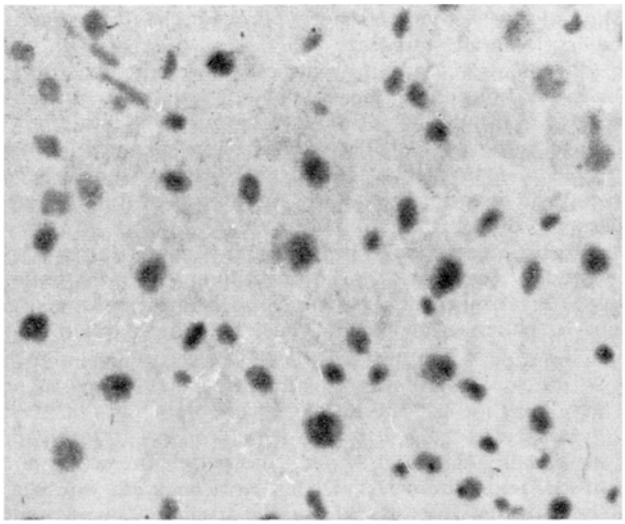

Fig. 2. Section of mouse liver incubated in potassium ferro-ferricyanide at pH 5.2 demonstrating staining of nuclei of liver cells. × 135.

nuclei was specifically delineated with non-staining of the parachromatin. There was no cytoplasmic staining. The staining appeared at 6 h but became more intense with further incubation with maximum staining at 19 h (Figures 1–3).

Discussion. The cause for the highly selective staining of cell nuclei of various tissues by the potassium ferro-ferricyanide is not clear. We discovered this phenomenon entirely by serendipity. The incubation of the tissues in the acid potassium ferro-ferricyanide solution was one of our controls in our recently completed study of intracellular exonuclear and endonuclear phosphodiesterases. This investigation was facilitated by our recent synthesis of 2 highly specific substrates for phosphodiesterases and DNases[8]. These substrates are 5-bromo-4-chloro-3-indolyl-thymidine-3′-phosphate and 5-bromo-4-chloro-3-indolyl-thymidine-5′-phosphate. The blue staining of the nuclei

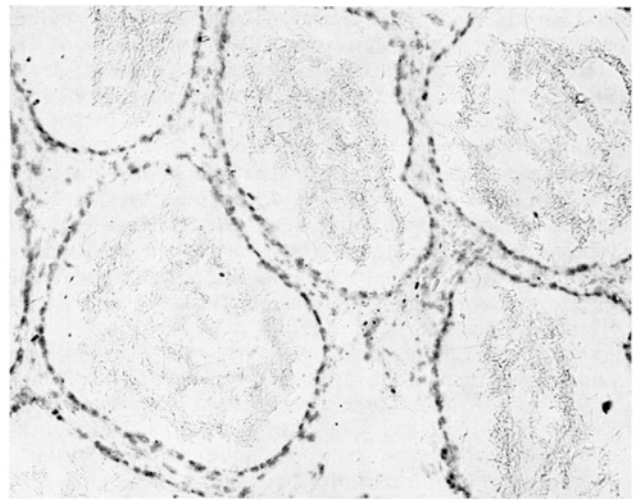

Fig. 3. Section of rat epididymis incubated in potassium ferro-ferricyanide at pH 5.2 demonstrating staining of nuclei of cells lining the tubules of the epididymis. × 90.

is most likely staining of iron compounds in the nuclei. The stain is extremely similar to the TURNBULL blue method for hemosiderin[15] and MALLORY's[16] or GOMORI's[17] histochemical method for cytoplasmic iron. Thus, we believe that certain iron containing substances present in cell nuclei have an affinity for potassium ferro-ferricyanide solution at pH 5.2 with the development of selective blue nuclear staining. One must also consider that DNA may have an affinity for ferro-ferricyanide[18].

Zusammenfassung. Frischgefrorene Gewebesektionen verschiedener Gewebe wurden in Kalium-Eisen-Cyanid bei pH 5,2 inkubiert; dabei wurde eine präzise und einzigartige Färbung nur der Zellnuklei beobachtet. Die Ursache dieser selektiven Kernfärbung ist nicht verständlich, doch stellt sie höchstwahrscheinlich eine Färbung von Ferro-Verbindungen in den Nuklei dar.

P. L. WOLF, J. P. HORWITZ,
JANICE VAZQUEZ
and ELISABETH VON DER MUEHLL

Detroit Institute of Cancer Research Division of the Michigan Cancer Foundation, Department of Pathology, Wayne State University, School of Medicine, and Detroit General Hospital, Detroit (Michigan 48226, USA),
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