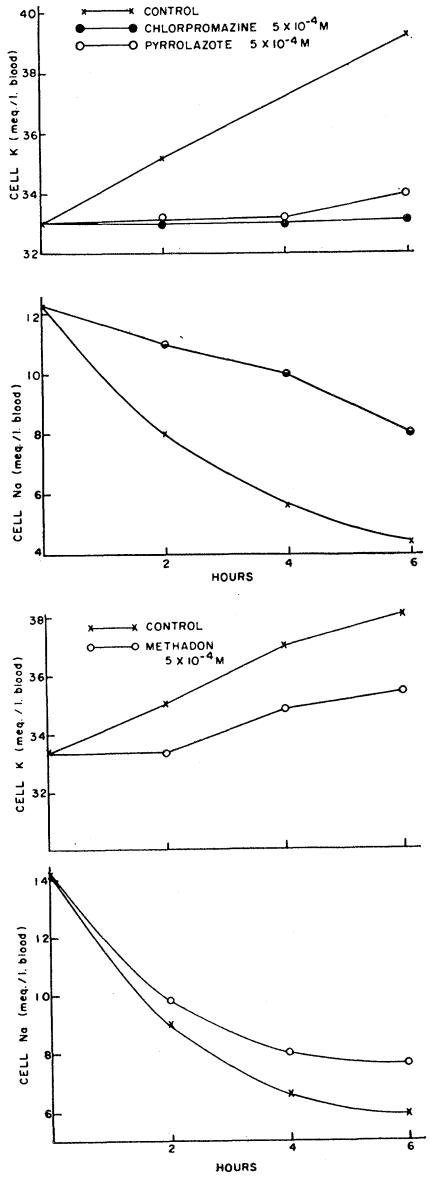

Fig. 1. Erythrocyte sodium and potassium plotted against time.

erythrocytes lose potassium and gain sodium. On warming to 37°C in the presence of the appropriate substrate, potassium is replaced in, and sodium removed from, the cell (*2*). There is no proof at present that the mechanism for active transport of cations is the same in all tissues, but it is likely that the fundamental process is, at least, similar.

The action of certain drugs that affect the central nervous system has been tested on the transport of sodium from, and potassium into, erythrocytes of blood that had been refrigerated for several days and then incubated at 37°C.

Freshly drawn human blood was defibrinated by shaking it with glass beads under sterile conditions and was stored in the refrigerator at about 5°C for 3 to 5 days. During this time, the erythrocytes lost potassium and gained sodium. On removal of the blood from the refrigerator, glucose (1 mg/ml blood) was added to it; sodium chloride solution (0.9 percent) was added to the control suspension (0.1 ml/ml blood); and the drug, which was dissolved in sodium chloride solution (0.9 percent), was added to the experimental suspensions. The blood was incubated at 37°C, and, at intervals of about 2 hours, 1-ml samples were removed and centrifuged. The plasma was removed from each sample, and the cells were washed with sucrose solution (9.8 percent) and hemolyzed by the addition of distilled water. The protein was precipitated by adding trichloroacetic acid to the hemolyzate to give a final concentration of 5 percent. The filtrate was used for the determination of sodium and potassium, using the Beckman DU spectrophotometer with flame attachment.

The drugs that were tested on the extrusion of sodium from, and uptake of potassium by, red cells were morphine sulfate ($10^{-3}M$), pentobarbital sodium ($2 \times 10^{-3}M$), methadone (*3*) (2.5 and $5 \times 10^{-4}M$), cocaine ($3 \times 10^{-4}M$), Pyribenzamine (*4*) ($3 \times 10^{-4}M$), chlorpromazine (*5*) (2.5 and $5 \times 10^{-4}M$), and Pyrrolazote (*6*) ($5 \times 10^{-4}M$). Three of these drugs—namely, methadone, chlorpromazine, and Pyrrolazote—inhibited both sodium and potassium transport. The results of a typical experiment are shown graphically in Fig. 1. This effect is similar to that described by Schatzmann (*7*) and by Glynn (*8*) for certain cardiac glycosides. The other drugs had no effect under our experimental conditions.

The concentrations of methadone that were used are sufficient to inhibit glycolysis and carbohydrate oxidation by brain tissue (*9*). However, the concentrations of the phenothiazine drugs that were used in these experiments had no effect on either glycolysis or oxidation of glucose by brain tissue. Thus, inhibition of transport cannot be correlated with inhibition of glycolysis in the case of the phenothiazine drugs.

The concentrations of phenothiazine drugs that were used in these experiments to inhibit ion transport are several times greater than those required to inhibit hemolysis by lysolecithin (*10*).

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4. Pyribenzamine hydrochloride, 2-[benzyl(2-dimethylaminoethyl)amino]-pyridine hydrochloride.
5. Chlorpromazine, 10-(*a*-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride.
6. Pyrrolazote (Reg. U.S. Pat. Off.) (pyrathiazine, Upjohn), 10-[2-(1-Pyrrolidyl)ethyl]-prenothiazine hydrochloride.
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25 November 1955

Enzymatic Detoxification Mechanism for Viadril

The structural similarity of the steroid anesthetic, Viadril (*1*) (pregnan-21-ol-3,-20-dione 21-hemisuccinate) (*2*), to substrates of several enzymes currently under investigation (*3–5*) has suggested that related reactions may be involved in its detoxification. Thus, Viadral might be reduced at the 3-position (reaction 1), and then be conjugated as the glucuronide (*6*). Alternatively, the following sequence of reactions may occur: esterase action on Viadril to produce succinate and pregnan-21-ol-3,20-dione (reaction 2), reduction of the steroid moiety catalyzed by 3α-hydroxysteroid dehydrogenase (reaction 3), and conjugation. These possibilities have been investigated, utilizing enzyme systems (*7*) partially purified from rat liver. The reactions examined were

$$\text{Viadril} + \text{DPNH} + \text{H}^+ \rightleftharpoons \text{pregnane-3}\alpha,\text{21-diol-20-one 21-hemisuccinate} + \text{DPN}^+ \quad (1)$$

$$\text{Viadril} + \text{H}_2\text{O} \rightarrow \text{Pregnan-21-ol-3,20-dione} + \text{succinate} \quad (2)$$

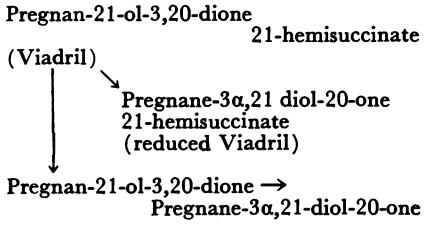
$$\text{Pregnan-21-ol-3,20-dione} + \text{DPNH(TPNH)} + \text{H}^+ \rightarrow \text{pregnane-3}\alpha,\text{21-diol-20-one} + \text{DPN}^+\text{(TPN}^+\text{)} \quad (3)$$

Reaction 1 was reversibly catalyzed by an ammonium sulfate fraction of rat liver that, when it was added to Viadral and DPNH, caused a rapid oxidation of the reduced pyridine nucleotide as measured in the spectrophotometer at 340 mμ. TPNH did not function in this reaction. When 3α-reduced Viadril (pregnane-3α, 21-diol-20-one 21-hemisuccinate) was incubated with the enzyme preparation and DPN^+, a prompt reduction of the pyridine nucleotide was observed. With 3β-reduced Viadril as substrate, DPN^+ was not reduced. It is of interest to note that reaction 1 was also catalyzed by αBAD (*3*) that was obtained from an organism isolated by enrichment culture. In the presence of DPNH, this enzyme reversibly reduces 3-ketosteroids with a terminal carboxyl group. Viadril was reduced to 3α-reduced Viadril by the enzyme at 40 percent of the rate of the reduction of ketocholanic acid.

Extracts of rat-liver acetone powder contained an esterase activity that catalyzed the hydrolysis of Viadril to succinate and pregnan-21-ol-3,20-dione (reaction 2). The enzyme activity was followed by measuring the unhydrolyzed ester that remained at the end of the incubation period, using the hydroxamic acid method of Baggett *et al.* (*8*). The steroidal hydrolysis product served as a substrate for reaction 3—that is, reversible reduction by reduced pyridine nucleotide in the presence of 3α-hydroxysteroid dehydrogenase from rat liver (*4*).

All the products of the reactions described were identified chromatographically, using modifications of the solvent systems of Bush (*9*). Calculation of the rates of the individual reactions, based on the assay conditions used here, yield the following values in micromoles of product formed per hour per gram of rat liver: reduced Viadril, 8; pregnan-21-ol-3,20-dione 21-hemisuccinate, 23; pregnane-3α, 21-diol-20-one, 15.

These findings are consistent with the following scheme for the detoxification of Viadril and predict the formation of conjugated forms of pregnane-3α, 21-diol-20-one 21-hemisuccinate and pregnane-3α, 21-diol-20-one as excretory end-products.



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References and Notes

1. Viadril is a trade name of the sodium salt of pregnan-21-ol-3,20-dione 21-hemisuccinate. Reduced Viadril refers to pregnane-3α,21-diol-20-one 21-hemisuccinate. Generous amounts of these compounds, as well as other steroids used here, were made available to us by the interested cooperation of G. D. Laubach and S. K. Figdor of Chas. Pfizer and Company.
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7. The following abbreviations are employed: DPN and TPN, di- and triphosphopyridine nucleotide, respectively; DPNH and TPNH, the reduced nucleotides; BAD, bile acid dehydrogenase.
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8 December 1955

Simple Procedure for Identification and Rapid Counting of Mast Cells in Tissue Sections

Early in an investigation of the distribution and numbers of mast cells in histologic sections of certain mammalian tissues and organs, it became evident that the microtechnical procedures in current use were not satisfactory. A review of 68 recent papers dealing with the occurrence of mast cells in tissue sections and spreads revealed that in only 7 instances were cell counts made that were susceptible to statistical analysis. Subjective impressions were frequently reported in lieu of numerical data, a fact deplored by Devitt, Pirozynski, and Samuels (*1*), who summarized the matter as follows: "Although numerical counting of tissue mast cells is difficult, and not an ideal method of investigating their response to various stimuli, their capricious distribution renders simple estimation of numbers by impression alone worthless."

There are two principal defects in the methods currently employed for the histologic demonstration of mast cells. In a surprisingly large proportion of the studies reviewed (38 percent), aqueous fixatives such as 10-percent formalin, Bouin's fluid, and Helly's fluid were used. These fixatives are notoriously unreliable for the preservation of mast cell granules, which are water-soluble in most species (*2*). Of even greater consequence was the widespread use of unbuffered aqueous solutions of basic thiazin dyes, most commonly toluidine blue. These dye solutions stain not only the mast cells but also the surrounding tissues, often to a very considerable extent (Fig. 1). As a result, in many instances the mast cells can be identified only with a high-power objective lens. Rapid and accurate counting is rendered difficult by the lack of differentiation, and it is especially troublesome in strongly basophilic organs such as the pancreas and liver.

To eliminate these difficulties, the following histologic procedure (*3*) was developed on the basis of theoretical considerations and practical experience.

1) Thin slices of fresh tissue (3 to 5 mm thick) are fixed in the following solution for 18 to 24 hours: formalin, 10 ml; 95-percent alcohol, 90 ml; and calcium acetate, 1 g. Specimens may be left in the fixative for at least 1 week without apparent harm.

2) After fixation, the tissues are washed in two changes of 95-percent alcohol (1 hour each). Paraffin sections (8 to 12μ thick) are subsequently prepared in the routine manner.

3) The mounted sections are deparaffinized, run through absolute to 95-percent alcohol, and then are stained in the following solution for 1 hour at room temperature: toluidine blue, 0.25 g; 70-percent alcohol, 100 ml; concentrated HCl, 0.5 ml. The dye solution should be filtered before using.

4) After the tissue sections are stained, rinse briefly in 95-percent alcohol containing 0.5-percent HCl to remove the excess dye (that is, until the sections are grossly colorless). Counterstain lightly by dipping in 0.01-percent eosin in 95-percent alcohol. Clear and mount in a resinous medium in the usual manner.

The results of this procedure are that mast cells and cartilage matrix are colored deep blue against a pale pink background (Fig. 2). The mast cells can readily be seen with the 10× objective lens. Satisfactory results have been obtained with sections of tongue, lung, liver, duodenum, spleen, and pancreas from the mouse, rat, bat, dog, and monkey.

The staining procedure presented here is based on the nonspecific binding of toluidine blue, a basic dye, with the acidic components in paraffin sections of tissues

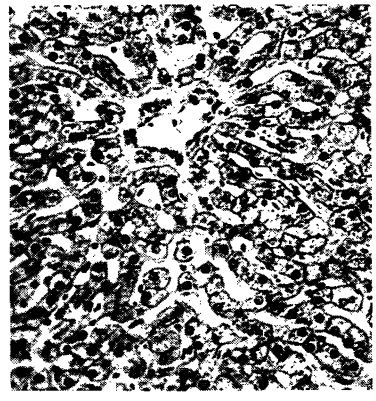

Fig. 1. Low-power photomicrograph of a section of dog liver stained with 0.25-percent aqueous toluidine blue. Note that the mast cells are obscured by the concurrent staining of the nuclei and of the cytoplasmic granules in the hepatic parenchymal cells.

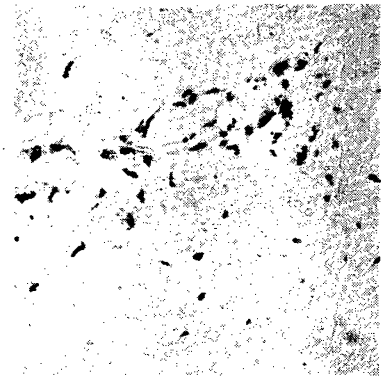

Fig. 2. Photomicrograph of a section parallel to that shown in Fig. 1, but stained by the procedure described in this report. Note that the mast cells stand out in sharp contrast to the lightly stained background.