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.

Pregnan-21-ol-3,20-dione 21-hemisuccinate (Viadril) → Pregnane-3α,21 diol-20-one 21-hemisuccinate (reduced Viadril)

Pregnan-21-ol-3,20-dione → Pregnane-3α,21-diol-20-one

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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.

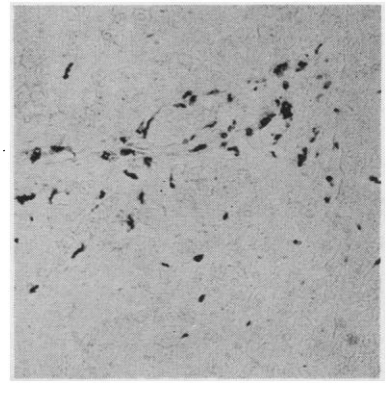

Fig. 2. Photomicrograph of a section parallel to that shown in Fig. 1, but stained by the procedure described in this report. Note that the mast cells stand out in sharp contrast to the lightly stained background.

that have been fixed in alcoholic formalin. Hence staining is not limited to the cytoplasmic granules of the mast cells. Because of the low *p*H of the dye solution, however, the dye binds with only the most strongly acidic tissue components, principally the sulfonated mucopolysaccharides. The only material in the tissues we have examined other than the mast cell granules, which is deeply stained, is cartilage matrix. In some organs that have a very high content of cytoplasmic ribonucleic acid or that have numerous dense nuclei, weak staining with the toludine blue may occur. Because there is little likelihood of confusion, the method can be considered, for practical purposes, to be highly selective for mast cells. It should also be noted that an acid dye is used as a counterstain only as a matter of convenience to facilitate the identification of the various tissues and organs on the slides.

The staining schedule given was deliberately designed to overstain the mast cells in order to obtain the greatest contrast with the surrounding tissues, and thus to make counting easier. If desired, greater cytologic detail can be obtained simply by shortening the period of staining in step 3. In order to make cell counts rapidly, the low-power objective lens may be used. To prevent confusion and in the interests of accuracy, however, scrupulous cleanliness must be observed during the preparation of the slides, especially in preventing the accumulation of dust particles on newly mounted sections during drying of the slides.

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Role of Teachers in Scholarship Programs

As a local committee, concerned with improving science teaching in the secondary schools as one means of alleviating the present critical shortage of scientists and engineers, we feel that one aspect of our findings should be brought to the attention of *Science* readers. A complaint frequently voiced by high-school science teachers concerns the burden of noninstructional duties they are expected to bear. In addition to the records and forms required by various local, state, and federal agencies, these teachers receive a multitude of questionnaires, data sheets, and various other types of requests for information from many public and private organizations. It seems unfortunate, to say the least, that many of the requests come from well-intentioned groups that are working toward improvement of our educational system.

The citation of a single example will, perhaps, serve to emphasize our point. The National Merit Scholarship Corporation, financed by grants from foundations and industry, recently initiated the largest nongovernmental scholarship program in the history of American education. In this program, about 60,000 seniors, selected from 10,000 high schools, were given a 3-hour competitive examination, after which 4000 students were selected as semifinalists and given the scholastic aptitude test of the College Entrance Examination Board. The 2000 scoring highest in the latter test will become finalists, and from this group approximately 400 will be awarded scholarships ranging from $100 to $2000 based on need. If the average cost of a 4-year scholarship including a "cost of education" grant to the college is $6000 [*Science* **122**, 508 (1955)], then the annual total value of the 400 scholarships would be $600,000. Such a contribution is truly impressive and, at first glance, seems richly rewarding for all concerned. Superior students receive recognition and financial aid, colleges fortunate enough to be selected by these superior students likewise receive recognition as well as financial assistance, while the donors receive favorable publicity not only in connection with the national interest aroused by the program but also through donor-identity which is maintained with all scholarships.

One group, however, seems to have been completely overlooked. The high-school principals and teachers who were "invited" to participate in the program undoubtedly should receive recognition for stimulating and training the superior students who are selected for scholarships. Even if this is denied on the argument that the teachers are merely carrying out their regular duties, they should, at least, receive credit for the role they play in the scholarship program. On the basis of experience in local high schools, we have attempted to calculate the number of man-hours that principals and teachers must contribute in order to participate effectively. We estimate that completion of entry forms, conferences, and record checking to select the most promising students and administration of the first screening examination required 6 hours for each of the 60,000 examinees, or a total of 360,000 man-hours. The time required to compile and submit the information required for the 4000 semifinalists and 2000 finalists is difficult to estimate but would probably bring the total close to a half-million man-hours. Assuming that the teachers' time is worth at least $1 per hour, their contribution would be nearly equal to the total value of the scholarships. Even if these estimates, which are admittedly rough, were reduced by half, the teachers' contribution would still be tremendous.

We wish to make it clear that this statement is not intended as a specific criticism of the National Merit Scholarship Program. All of the high-school principals and teachers contacted felt that the program was well worth the time required for participation. Several pointed out that less valuable programs of various types made more excessive demands on their time. We hope that the example cited may encourage administrators of such programs to examine their procedures with the view of reducing to a minimum the demands placed on secondary-school personnel. In the case cited, we should also like to suggest that some concrete form of recognition be awarded to those high schools that have scholarship winners. This might be done by awards to the teacher or teachers that the winners designate as most responsible for their success, or, if such a plan is not feasible, an unrestricted award to the school for the purpose of improving teaching might be made. We believe that such awards would serve to draw public attention to the important contributions made by the secondary-school teachers.

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Infectivity of Tularemia Applied to Intact Skin and Ingested in Drinking Water

In his book on tularemia, Simpson described in the following words Francis' demonstration of the penetration by *Bacterium tularense* (*Pasteurella tularensis*) of the unbroken skin: "Francis dropped onto the unbroken skin of normal guinea pigs, suspensions of splenic tissue of guinea pigs recently dead of tularemia; the guinea pigs were dead in five days; at autopsy they showed the