ably be suggested. Tissue hypoxia can be considered as resulting from circulatory modifications during hibernation. Lyman and O'Brien(11) indicate a uniform vasoconstriction, and Bullard and Funkhauser(12) report marked reduction in blood flow in numerous tissues and organs in hibernating ground squirrels. There is also evidence of increased catecholamine content in tissues of hibernating squirrels(13), and nor-adrenalin and other catecholamines have been implicated as radio-protective agents(14). At this time the question of mechanisms responsible for the decreased radio-sensitivity is still under investigation.

Summary. The basic conclusion of this report is that irradiated hibernating ground squirrels do not respond as "active" animals following arousal as has been hitherto reported, rather, there is a protective effect relatable to hibernation. The hibernators have a longer "mean survival time" and an increased $LD_{50(30)}$.

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The Indigogenic Reaction for Histochemical Demonstration of Sulfatase.* (31966)

PAUL L. WOLF, JEROME P. HORWITZ, JANICE VAZQUEZ, JONATHAN CHUA, MYUNG SOOK YUN PAK, AND ELISABETH VON DER MUEHLL
(Introduced by A. S. Prasad)
Department of Pathology, Wayne State University School of Medicine and Detroit Insitute of Cancer Research, Detroit, Mich.

Previous work in this laboratory described the application of the indigogenic principle to the histochemical demonstration of leucine aminopeptidase(1), β-glucosidase (2), β-galactosidase(3), N-acetyl-β-glucosaminidase(4), and alkaline and acid phosphatase(5). In the present study we have extended this principle to histochemical localization of sulfatase, utilizing 5-bromo-4-chloro-3-indolyl sulfate. The substrate was synthesized according to methods described recently by Horwitz and coworkers(6,7). The substrate offers the advantage of a precise enzyme localization with no or very slight diffusion. Moreover, the substrate affords a simple and direct method for demonstration of hydrolytic enzymes without the need for a coupling reaction. This compound yields a blue-green deposition of indigo.

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Methods. Tissues from the mouse, rat, guinea pig, and human 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 O.C.T. compound (optimal cutting temperature), 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 one minute until the embedding medium was frozen and became the proper consistency for cutting 6 μ sections at —20°C.

Cold acetone fixed or cold neutral formalin-fixed sections were incubated for 2 hours in solutions containing 5-bromo-4-chloro-3-indolyl sulfate. The incubating solution employed for sulfatase is as follows: 14.0 ml Tris buffer, pH 6.1, 0.05 M; 1.0 ml 5-bromo-4-chloro-3-indolyl sulfate, 7.2 mg/ml H_2O, 0.00031 M (final concentration); 1 ml, $MgCl_2$, 0.005 M (final concentration). The total volume in Coplin jar is 16 ml. After incubation the slides were washed briefly in tap water and mounted in glycerol gel for microscopic examination.

Results. Tissues from all major organs of rats, guinea pigs, mice and humans were examined for sulfatase activity. The indolyl substrate gave a final blue-green indigo at the site of enzyme localization. Substances which inhibited the histochemical reaction were sulfite, cyanide and fluoride. Calcium and magnesium accelerated the histochemical reaction.

Our histochemical observations indicated that sulfatase activity was present in the normal rat, human, mouse and guinea pig with tissues of the rat having the highest activity, and the guinea pig the least. Highest activity was present in the liver, kidney, pancreas and adrenal (Fig. 1). Lesser activity was found in salivary gland, gastrointestinal tract, reticular cells of reticuloendothelial system, ovary, testis and central nervous system. There was no activity in skeletal muscle, heart and thyroid. The deposition of the blue-green indigo was finely granular and located at cytoplasmic lysosomal sites.

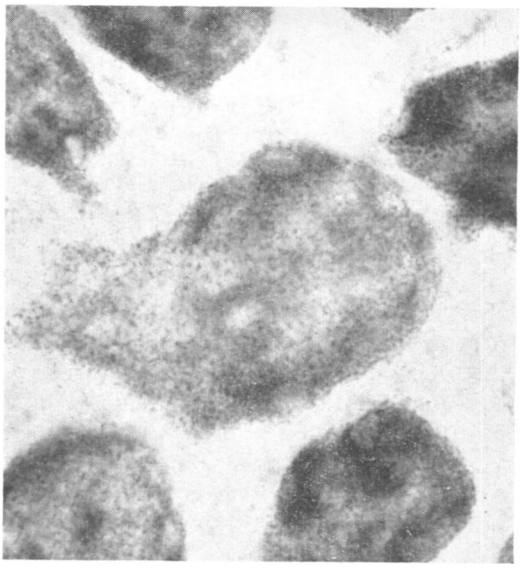

FIG. 1. Section of rat kidney stained for sulfatase utilizing 5-bromo-4-chloro-3-indolyl sulfate demonstrating granular precipitate of indigo at lysosomal sites in the renal tubular cytoplasm. ×-1200.

Discussion. This paper describes the histochemical demonstration of sulfatase utilizing a new indolyl chromogen. We obtained precise enzyme localization with little or no diffusion.

Up to the present time histochemical procedures for the demonstration of sulfatases have been unsatisfactory(8). Previous methods have employed simultaneous coupling azo dye(9), post coupling azo dye(10), and naphthol AS-sulphate technique(11).

A previous indoxyl sulphate procedure employed by Sunder utilized the disulphate ester of leuco-indigo as a substrate; however, the histochemical reaction may have resulted from the presence of oxidases(12). There have been indications stipulating the need for further work in the application of the indigogenic principle to the localization of sulfatase activity(13,14). Failure to expand this area to date is in all probability due to the difficulty in the requisite substrate-synthesis.

Summary. The indigogenic principle has been extended to the histochemical demonstration of sulfatase. The advantage of this substrate is that it affords a precise enzyme

localization with no or very slight diffusion and also a simple and direct method for demonstration of this enzyme without the need for a coupling reaction.

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Determination of Anti Group A Streptococcal Polysaccharide Antibodies in Human Sera by an Hemagglutination Technique.* (31967)

ISRAËL GOLDSTEIN AND RENÉ CARAVANO (Introduced by D. Kritchevsky)
Rheumatic Fever Research Laboratory, International Children's Centre, Paris 75(16), France

Although there are several excellent tools for serologic diagnosis of Group A streptococcal infection (such as antistreptolysin, antihyaluronidase, antistreptokinase), it is apparent that all current serological reactions are related to the extracellular antigens of Group A streptococcus. With regard to somatic antigens, only the anti M antibodies have been studied to any extent, because of the interest in these type-specific antibodies for the study of immunity. It may be supposed that a reliable technique for determination in human sera of antibodies against the group-specific polysaccharide (C antigen) would be useful in acquiring knowledge of the patterns of Group A streptococcal infection, since it would show an immunologic response to a somatic component common to the various serological types. Several studies dealing with this problem have been published recently (1,2,3,4). The main difficulty is that, if the antigen is in a crude extract, it is mixed with other antigens, so that the specificity of the reaction becomes uncertain; and on the contrary, a highly purified polysaccharide is so inert that an appropriate serological technique is very difficult to set up. We recently devised a technique using a highly purified polysaccharide labelled with ^{14}C(7). This technique seems accurate and reliable but needs expensive equipment, particularly a scintillation meter.

The present paper describes a new method for determination of these antibodies which does not present technical difficulties and seems to give accurate and reliable results.

Material and methods. 1. Hemagglutination reaction. The polysaccharide extracted from the cell wall of Group A streptococcus was

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