pound also has an adrenolytic action like chloropromazine because of its phenylpiperazine moiety. Bovet *et al.*(12) have shown that 1-phenylpiperazine is weakly adrenolytic, and Archer *et al.*(13) have reported that the indole-arylpiperazines have adrenolytic properties. Thus, the CNS depression may be analogous to that ascribed to chloropromazine and unrelated to depletion of norepinephrine. The excitation exhibited by monoamine oxidase inhibited animals given either reserpine or α-methyl-meta-tyrosine has been explained by the release of norepinephrine(14). The CNS depression produced with Win 18501-2 was not reversed by pretreatment with a monoamine oxidase inhibitor. The adrenolytic properties of the compound may also account for this finding.

Summary. Administration of an indolylalkyl-arylpiperazine (Win 18501-2) to rats depletes the norepinephrine content of heart and brain without affecting brain serotonin. Maximum depletion was observed within 3 hours with return to normal values within 8 hours. These findings suggest that the turnover of heart and brain norepinephrine in the rat is very rapid.

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Tetrazolium Salts and Identification of *Corynebacterium diphtheriae.**† (27711)

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Media containing tellurite have been used extensively for detection and isolation of *Corynebacterium diphtheriae*(1,2,3) because this species reduces potassium tellurite(1,2). This is generally considered a valuable tool, even though certain other bacteria react somewhat similarly(4,5,6).

Tetrazolium salts are valuable reagents in the study of dehydrogenase systems and are superior to tellurium salts for this purpose because of their higher redox potentials(7). One of them, nitro blue tetrazolium (NO_2BT) [(-2, 2'-di-p-nitrophenyl-5, 5'-diphenyl-3, 3'-3, 3'-dimethoxy-4, 4', biphenylene) ditetrazolium chloride](8), possesses several remark-

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† The various tetrazolium salts used, with the exception of TNBT, may be purchased from Sigma Chemical Co., St. Louis, Mo. Potassium tellurite, 1% solution, was obtained from Difco Lab, Detroit, Mich.

TABLE I. Reactivity of *C. diphtheriae*, Hammond Strain, with Tetrazolium Salts.

Salt	Reaction at 2 min.
NO_2BT	Jet black
TNBT	Black
INT	Black to gray*
BT	Negative*
NT	"
TPT	" †
Tellurite	" †

* Turned black at 1 hr.

† Usually turned black after 120 min.

Concentration of tetrazolium salts was 5 mg/ml. Tellurite was 1%. Data derived from 24 hr cultures on trypticase soy agar with 5% human blood. Colonies were flooded with 1 or 2 drops of reagent solution and read at 2 min.

Note that order of reactivity follows closely the redox potential of the reagents(7). No value for the redox potential of TNBT is available.

able properties, the most important being the ease with which it is reduced. This suggested to us that this compound might be useful for identifying *C. diphtheriae*.

The purpose of this investigation was a comparative study of the ability of certain bacteria and yeasts to reduce some tetrazolium salts. It was predicted that these compounds, notably NO_2BT, would yield reactions of a higher degree of intensity than potassium tellurite, and that this may be useful for identifying *C. diphtheriae*.

Observations. A disadvantage of incorporating tellurium or tetrazolium salts into the medium is that they inhibit bacterial growth in varying degree(9,10,11). Therefore, we decided to eliminate the reacting agent from the medium. After growing bacterial colonies on trypticase soy agar with 5% human blood for 18 to 24 hours, the reducing properties of the colonies were tested by means of the following reagents: potassium tellurite, 10 mg per ml of distilled water; Nitro BT; blue tetrazolium (BT) [2, 2′, 5, 5′-tetraphenyl-3, 3′-(3, 3′-dimethoxy-4, 4′-biphenylene) ditetrazolium chloride]; neotetrazolium (NT) [2, 2′, 5, 5′-tetraphenyl-3, 3′-(4, 4′-biphenylene) ditetrazolium chloride]; iodonitrotetrazolium (INT) [2-p-nitrophenyl-3-p-iodophenyl-5-phenyltetrazolium chloride]; triphenyltetrazolium (TPT) [2, 3, 5-triphenyltetrazolium chloride]; tetranitro blue tetrazolium (TNBT) [2, 2′, 5, 5′-tetra-p-nitrophenyl-3, 3′-(3, 3′-dimethoxy-4, 4′-biphenylene)-ditetrazolium chloride](7,12) all at the concentration of 5 mg per ml of distilled water.

A toxigenic strain of *C. diphtheriae* (Strain Hammond, 1956) isolated in this laboratory from a patient was used in the preliminary studies. Plate cultures were incubated for 24 hours, then reactivity of colonies was tested by covering an area of the culture with a drop of a tetrazolium salt or potassium tellurite. The reaction was timed with a stop watch. Results are summarized in Table I. As predicted, NO_2BT provided the most intense reaction. A few seconds after the NO_2BT solution was added, colonies of *C. diphtheriae* turned gray, appeared dark blue in about 20 seconds, and became jet black in 2 minutes by reflected or transmitted light. The value of NO_2BT for identifying *C. diphtheriae* seemed apparent to us.

To establish the specificity of the test with NO_2BT, a number of bacterial species were tested, including another strain of *C. diphtheriae* isolated in this laboratory (Strain Wood, 1958), and the following strains of *C. diphtheriae* obtained from the American Type Culture Collection(13): ATCC #6928, 8024, 8028, 296, 9059, 9456, 8032, and 11049. All of these strains of *C. diphtheriae* reacted very much like the Hammond Strain. Results are tabulated in Table II.

It was observed that colonies of some strains of nonpathogenic diphtheroids, *Staphylococcus aureus, S. albus, Sarcina* and *Neisseria* also reduce NO_2BT rapidly. However, the reaction of all strains of *C. diphtheriae* tested invariably was more intense and prompt. Colonies of cocci can easily be identified by examination of stained smears. The various species of *Corynebacterium* must still be differentiated by means of the usual biochemical and toxigenicity tests. The negative results with all the strains of *Streptococcus pyogenes* deserve special comment, since this test may help to rule out diphtheria in cases presenting membranous lesions in the throat.

Discussion. The reaction of the bacteria with potassium tellurite and tetrazolium salts seems to be enzymatic(5,14). We observed that 40% Formalin for 30 minutes will prevent the color reaction.

These results indicate that NO_2BT pos-

TABLE II. Reactivity of Microorganisms with NO_2BT.*

Species	No. Strains tested	Color Reaction at 2 min.
Corynebacterium diphtheriae	10	Black
C. pseudodiphtheriticum	2	"
C. acnes	4	"
C. ulcerans	1	Gray
C. xerosis	1	Gray
Sarcina lutea	3	Black
Staphylococcus aureus	44	Black to gray†
S. albus	88	" " " †
S. citreus	1	Gray
Micrococcus sp.	2	Black
Neisseria flava	9	Black to gray†
N. sicca	6	" " " †
N. meningitidis	1	Neg.
Escherichia coli	77	Gray
E. freundii	1	"
Proteus mirabilis	30	Purple
P. vulgaris	5	"
Pseudomonas aeruginosa	28	Purple to neg.
Candida albicans	8	Gray†
Aerobacter sp.	25	Gray to neg.
Paracolobactrum coliforme	4	Gray
P. aerogenoides	1	" ‡
Flavobacterium sp.	3	Neg.
Hemophilus influenzae	4	Gray
Geotrichum candidum	2	Neg.
Rhodotorula sp.	1	Red
Streptococcus faecalis	18	Gray to neg.
S. viridans	35	Neg.
S. pyogenes	14	"
Diplococcus pneumoniae	28	" §
Peptostreptococcus sp.	1	"
Lactobacillus sp.	3	"
Clostridium perfringens	3	"
B. subtilis	1	"
Salmonella Group B	1	"
Serratia marcescens	1	"
Bordetella parapertussis	1	"
Mycobacterium kansasii	1	"
Nocardia asteroides	3	"
Nocardia sp.	2	"
Streptomyces albus	1	"

* Pure cultures were grown for 24 hr at 37°C on trypticase soy agar with 5% human blood, and colonies were then covered with 0.5% aqueous solution of NO_2BT; color reaction was recorded.

† Blackening after 10 min.

‡ Periphery of colony turned purple.

§ Delayed blackening occurred with 2 strains.

Note that NO_2BT withstands autoclaving at 120°C at 15 lb for 15 min.

sesses a similar range of reactivity to that of tellurite. Although tellurite has been considered a selective agent for demonstrating *C. diphtheriae,* it is the experience of many that other bacteria, notably micrococci, will also blacken with it(5,6,9). Advantages offered by the procedure herein reported are that it eliminates the electron acceptor from the medium, facilitating bacterial growth, and permits easy and earlier identification of *C. diphtheriae.*

Subcultures of the Hammond Strain, taken from colonies 10 minutes and 24 hours after exposure to NO_2BT, proved virulent to baby chicks and guinea pigs, but not to animals protected with an earlier injection of diphtheria antitoxin.

Smears obtained from blackened colonies and stained with a Gram's procedure showed that the bacterial morphology was not altered by the formazan deposit.

These observations indicate that the series of microorganisms studied may be separated into 2 main groups: (1) a small group in which fast and intense reaction was obtained in 2 minutes or less; this reaction was most prominent with *C. diphtheriae,* and (2) a larger series which showed a wide spectrum of reactivity ranging from a weak to negative reaction at 2 minutes. Some of the bacteria in the second group reacted after longer intervals.

It is suggested that reactivity of oxidative enzymes is the determining factor in the behavior of microorganisms toward electron acceptors.

The present study indicates that nitro blue tetrazolium (NO_2BT) should have application in detection of *C. diphtheriae.*

Summary. A simple method for detection of *C. diphtheriae* by means of nitro blue tetrazolium (NO_2BT) is presented. This procedure is based on the marked reducing activity of *C. diphtheriae* and the very sensitive indicator properties of NO_2BT.

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Search for Virus in Human Malignancies. 2. *In vivo* Studies.* (27712)

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The findings in studies to recover viruses in cell culture systems from human malignant tissues have been recorded(1). This *in vitro* work was paralleled by *in vivo* studies in which newborn mice and hamsters were employed as the indicator system. The results obtained in the latter study are presented here.

Materials and methods. The collection and processing of *clinical specimens* and methods for preparation of *tissue cultures* from malignant tissues have been described(1). *Mice* of the ICR/Ha strain were maintained in these laboratories as a random bred strain. For breeding purpose, one male and one female were held as a monogamous family unit from 8 weeks to 10 months of age. The young were weaned at 21 days. The feed consisted of high fat (11%) breeder diet (Ralston Purina). The colony routinely proved free of Salmonella, ectromelia and other common mouse pathogens, and special work carried out in our laboratory indicated freedom from PVM and LCM viruses. The experimental mice and 700 breeder mice were tested and found free of hemagglutination-inhibiting (HI) antibodies for polyoma virus. Pregnant *hamsters* were obtained from Lakeview Hamster Colony, Newfield, N. J. During experimental use, the animal diet consisted of pelleted Rockland Mouse Diet supplemented twice weekly with green peanuts and kale or carrots. *Animal inoculation and observation.* Newborn mice less than 20 hours old were inoculated subcutaneously in the dorsal nuchal area with 0.05 to 0.1 ml of material. The mice were weaned at 21 days of age and the mother was discarded. Hamsters less than 20 hours of age were inoculated subcutaneously with 0.1 to 0.2 ml of sample. The animals were weaned at 18 to 21 days of age, and the mothers were discarded after bleeding for tests for polyoma antibody. During the first 60 to 90 days, each litter of mice or hamsters was housed individually in "sealed" filter cages(2) of special design which precluded airborne cross-infection between cages. All manipulations, such as feeding and palpation of animals, were performed on the individual cages inside a hood equipped with ultraviolet light, continuous exhaust, entrance and exit locks, and rubber sleeves and gloves. Autoclaved water bottles and water were used and the tube was inserted into the cage through a tight-fitting aperture. After the early critical 2- or 3-month period, animals were transferred to ordinary holding boxes with mesh screen lids. The sexes were not segregated. The animals were palpated at least once each week and, in addition, each cage was examined twice daily for dead animals,

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