(XI) and 5 ml. of *o*-dichlorobenzene heated in an oil bath at 155°. A clear solution was obtained after 5 min. A yellow solid separated on cooling; this was filtered and washed with benzene; yield, 0.20 g. (67%), m.p. 247–250°, raised to 248–251° on crystallization from toluene. By mixed melting point and infrared comparison, this was identical with an authentic sample.

Acknowledgement. The author is indebted to Miss J. L. Gove for spectral data and to O. E. Sundberg and his associates for microanalyses.

BOUND BROOK, N. J.

[CONTRIBUTION FROM THE DEPARTMENTS OF SURGERY AND CHEMISTRY, SINAI HOSPITAL OF BALTIMORE, INC., AND THE JOHNS HOPKINS UNIVERSITY, BALTIMORE, MARYLAND, AND THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF LONDON, ENGLAND.]

Preparation of Nitrotetrazolium Salts Containing Benzothiazole[1]

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In order to take advantage of the favorable influence for histochemistry of the *p*-nitrophenyl group at *N*-2 and the potential of chelating heavy metals by the thiazole-(2) group at *N*-3 in tetrazolium salts, a variety of mono and ditetrazolium salts incorporating these features were prepared. The simplest analogue (II) was found to chelate well and was reduced readily by dehydrogenase systems of mammalian tissues. Its redox potential was close to that of INT and Nitro-BT. The benzothiazole-(2) group in the C-5 position did not participate in chelation.

The discovery that a *p*-nitro group in the *N*-2 phenyl ring of tetrazolium salts confers favorable properties on the readiness with which they accept hydrogen from various dehydrogenase systems[2,3] and the discovery that a formazan containing a dimethylthiazole group at the N-3 position (MTT) chelates well with cobalt,[4,5] suggested to us that it would be worthwhile to prepare N-3 and C-5 benzothiazole derivatives of tetrazolium salts containing in addition a *p*-nitrophenyl group at N-2. It was also hoped that the remarkable substantive properties for protein in histochemical methodology[6] exhibited by Nitro-BT [2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene) ditetrazolium chloride] could be duplicated in benzothiazole analogues of dinitroditetrazolium salt. Although Nitro-BT and a 5-*m*-iodophenyl analogue of Nitro-BT have been used to demonstrate dehydrogenases with electron microscopy,[7] there should be an advantage to using nitrotetrazolium salts that yield formazans of high substantivity and capable of chelating metals of high atomic number. The development of such agents should make possible precise intramitochondrial localization of dehydrogenase activity with the electron microscope. For this purpose benzothiazole groups were introduced into the N-3 and C-5 positions and *p*-nitrophenyl groups were placed at N-2. Ditetrazolium salts were also prepared with benzothiazol and *p*-nitrophenyl groups, related to BT and Nitro-BT.

The formazan (I) was obtained by coupling *p*-nitrobenzene diazonium chloride with benzothiazolylhydrazone-(2) of benzaldehyde in the presence of alkali. Attempts to prepare I by coupling diazotized 2-aminobenzothiazole with *p*-nitrophenylhydrazone of benzaldehyde failed. Oxidation of I with *N*-bromosuccinimide in ethyl acetate[8] gave the tetrazolium bromide, which was converted to the corresponding chloride (II) by treatment with silver chloride. Oxidation of I with isoamyl nitrite and glacial acetic acid resulted in the formation of a tetrazolium salt (III) containing two nitroso groups.[9] Attempts to remove these nitroso groups with ethanolic hydrochloric acid[9] resulted in the formation of IV which did not react like a tetrazolium salt.

Similarly, benzothiazolylhydrazone-(2) of benzothiazole-2-aldehyde[10] on treatment with *p*-nitrobenzene diazonium chloride gave a formazan (V), which on oxidation with *N*-bromosuccinimide in ethyl acetate gave a tetrazolium bromide which was converted with silver chloride to the corresponding tetrazolium chloride (VI). Oxidation of V with isoamyl nitrite and glacial acetic acid gave a nitroso derivative (VII) which on treatment

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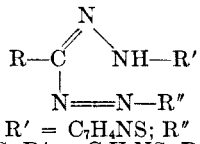
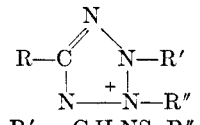
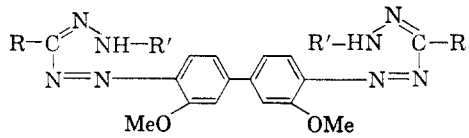


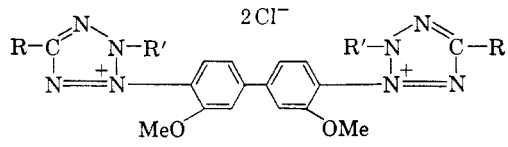
I. R = C_6H_5; R′ = C_7H_4NS; R″ = p-NO_2-C_6H_4
V. R = C_7H_4NS; R′ = C_7H_4NS; R″ = p-NO_2-C_6H_4
IX. R = C_7H_4NS; R′ = p-NO_2-C_6H_4; R″ = o-OMe-p-(m-OMe-C_6H_4)-C_6H_3
XIII. R = C_6H_5; R′ = C_7H_4NS; R″ = o-OMe-p-(m-OMe-C_6H_4)-C_6H_3



II. R = C_6H_5; R′ = C_7H_4NS; R″ = p-NO_2-C_6H_4
VI. R = C_7H_4NS; R′ = C_7H_4NS; R″ = p-NO_2-C_6H_4



X. R = C_7H_4NS; R′ = p-NO_2-C_6H_4
XIV. R = C_6H_5; R′ = C_7H_4NS



XII. R = C_7H_4NS; R′ = p-NO_2-C_6H_4
XV. R = C_6H_5; R′ = C_7H_4NS

with alcoholic hydrochloric acid gave a crystalline compound (VIII) which has not been identified.

The C-5 benzothiazole analogue of Nitro-BT was prepared from the p-nitrophenylhydrazone of benzothiazole-2-aldehyde[10] and tetrazotized 3,3′-dimethoxybenzidine at −20 to −35° as reported for Nitro-BT.[7] The resulting mixture of mono and diformazans (IX and X) were separated by extraction with benzene or methanol in a Soxhlet apparatus for a week. From the extracts, the monoformazan (IX) was obtained and purified by the usual procedure.[7] The diformazan (X) which remained in the thimble could be purified for analysis by crystallization from pyridine. It was much more soluble than the very insoluble diformazan from Nitro-BT.[2] Oxidation of X was effected with N-bromosuccinimide in dioxane and the resulting bromide (XI) was converted to the chloride (XII) with silver chloride.

The N-2 benzothiazole analogue of BT(XIV) was prepared from benzaldehyde, 2-benzothiazolylhydrazone and tetrazotized orthodianisidine. It was oxidized to the ditetrazolium bromide salt (XVa) with N-bromosuccinimide in chloroform and converted to the chloride (XV) with silver chloride. The nitroso acetate (XVI) was obtained on oxidation of XIV with isoamylnitrite.

In histochemical experiments with frozen sections of rat tissue, the benzothiazole tetrazolium salt (II) was very rapidly reduced by dehydrogenase systems as would be expected from its high position in Table I. The resulting formazan formed a black stable chelate with cobaltous ions which crystallized too readily for practical histochemical use. The other benzothiazole derivatives did not form chelates with cobalt under the conditions of the study. Investigation of other ions which do not inhibit the dehydrogenases significantly will be made.

EXPERIMENTAL

2-Benzothiazolyl-(2)-3-p-nitrophenyl-5-phenylformazan (I). Benzaldehyde, 2-benzothiazolylhydrazone (3.15 g.) was dissolved in tetrahydrofuran (250 ml.). p-Nitroaniline (1.72 g.) was suspended in 50% hydrochloric acid (9.0 ml.), and was diazotized with an aqueous solution of sodium nitrite (0.90 g.), at 0°. The diazotized solution was then added to the tetrahydrofuran solution at −20 to −25°. An aqueous solution of potassium hydroxide (20%) was added immediately in portions until the solution became alkaline to litmus. The solution turned deep blue. The solution was stirred for 4 hr. and was allowed to stand overnight at room temperature. It was diluted with a large volume of water, the precipitate was collected, washed with hot water, and crystallized from dilute tetrahydrofuran in shining violet needles, m.p. 204–205°. Yield (3.2 g.; 64%).

Anal. Calcd. for $C_{20}H_{14}O_2N_6S$: N, 20.89. Found: N, 21.20.

2-Benzothiazolyl-(2)-3-p-nitrophenyl-5-phenyltetrazolium bromide (IIa). The formazan (I) (0.50 g.) was refluxed with ethyl acetate (150 ml.), and filtered. To the filtrate was then added a solution of N-bromosuccinimide (0.23 g.) in ethyl acetate (20 ml.). On shaking for some time the color of the solution turned yellow. After the addition of a few drops of conc. aqueous hydrobromic acid, the solution was cooled to 0°. The yellow precipitate was collected (0.30 g.; 50%). It was crystallized by solution in hot water with the addition of a few drops of hydrobromic acid, in shining yellow plates, m.p. 215° dec. The bromide was sparingly soluble in water.

Anal. Calcd. for $C_{20}H_{13}O_2N_6SBr$: Br, 16.62. N, 17.47; Found: Br, 16.30. N, 17.30.

2-Benzothiazolyl-(2)-3-p-nitrophenyl-5-phenyltetrazolium chloride (II). The tetrazolium bromide (IIa) (0.10 g.) was refluxed in water (150 ml.) with freshly precipitated silver chloride for 10 hr. The mixture was filtered and the filtrate was evaporated on a steambath. The precipitate crystallized from a little water containing a few drops of hydrochloric acid in shining yellow plates, m.p. 190° dec. after shrinking at 180°. Yield (0.04 g.; 45%).

Anal. Calcd. for $C_{20}H_{13}O_2N_6SCl$: Cl 8.13. N, 19.24. Found: Cl, 8.40, N, 19.42.

Oxidation product of I *with isoamylnitrite* (III). The formazan (1.10 g.) was dissolved in boiling glacial acetic acid (50 ml.). The solution was cooled to room temperature, isoamylnitrite (5.0 ml.) was added, and the solution was reheated on a steambath until the color of the solution bleached to golden yellow. On cooling a yellow crystalline precipitate appeared. It was collected and washed with a little ether, yield 1.10 g. It crystallized from glacial acetic acid in yellow needles, m.p. 229–230° dec. Nitrogen analysis revealed two nitroso groups. The carbon and hydrogen analysis was not done because the specimen exploded during combustion analysis.

Anal. Calcd. for $C_{20}H_{11}N_6O_2S \cdot C_2H_3O_2 \cdot 2$ NO: N, 21.62. Found: N, 21.36.

This tetrazolium salt (III) could be reduced to the original formazan.

Anal. Calcd. for $C_{20}H_{14}O_2N_6S$: N, 20.89. Found: N, 21.31.

Compound IV. The tetrazolium salt (III, 50 mg.) was suspended in absolute alcohol (30 ml.). A stream of dry hydrochloric acid gas was passed for half an hour with external water cooling. The solution was refluxed on a steambath for half an hour and was concentrated to about 15 ml. On cooling very pale yellow crystals appeared, which crys-

TABLE I

COMPARISON OF POLAROGRAPHIC RESULTS WITH A VARIETY OF TETRAZOLIUM SALTS[a]
(E 1/2, *p*H 7.0, 22°)

Compound	Formula	Volts
II	2-Benzothiazolyl-(2)-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride	−0.04
Nitro-BT	2,2′-Di-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-5,5′-diphenyl ditetrazolium chloride	−0.05
VI	2-Benzothiazolyl-(2)-3-*p*-nitrophenyl-5-benzothiazolyl-(2)-tetrazolium chloride	−0.07
INT	2-*p*-Nitrophenyl-3-*p*-iodophenyl-5-phenyltetrazolium chloride	−0.09
4,5-MTT	3,5-Diphenyl-2-(4,5-dimethylthiazolyl-(2) tetrazolium bromide	−0.11
5-MTT	3,5-Diphenyl-2-(5-methylthiazolyl-(2)-tetrazolium bromide	−0.12
XII	2,2′-Di-*p*-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-5,5′-dibenzothiazolyl-(2)-ditetrazolium chloride	−0.15
BT	2,2′-Diphenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-5,5-diphenyl ditetrazolium chloride	−0.16
NT	2,2′-Diphenyl-3,3′-(4,4′-biphenylene)-5,5′-diphenyl ditetrazolium chloride	−0.17
TTC	Triphenyl tetrazolium chloride	−0.46
Tellurite		−0.95

[a] Acknowledgement for help in obtaining these results is due Mrs. B. Lamb, Research Department, Evershed and Vignoles Limited, London, England. Measurements were made with a Tinsley recording polarograph and a dropping mercury electrode. The tetrazolium salts were made up to a calculated concentration of 10^{-4} *M* in 0.1*M* phosphate buffer, *p*H 7.2, so that the final *p*H was 7.0.

tallized from alcohol in stout prismatic needles, m.p. 183° dec. The compound was insoluble in water, and it did not produce the dark color of formazan when a crystal of sodium sulphide was added to its solution in aqueous alcohol.

Benzothiazole-2-aldehyde-2-benzothiazolylhydrazone. (Va). This compound was prepared from equivalents by mixing alcoholic solutions of 2-hydrazinobenzothiazole (5%) and benzothiazole-2-aldehyde (20%). It crystallized from dioxane in yellow needles, m.p. 270° dec. after shrinking at 265°.

Anal. Calcd. for $C_{15}H_{10}N_4S_2$: N, 18.06. Found: N, 17.87.

2-Benzothiazolyl-(2)-3-p-nitrophenyl-5-benzothiazolyl-(2)-formazan (V). The hydrazone (Va, 1.24 g.) was dissolved in a mixture of tetrahydrofuran (40 ml.) and dimethylformamide (15 ml.). The solution was cooled to −20 to −30°. A diazotized solution of *p*-nitroaniline (0.55 g.) was added and the solution was made alkaline by the addition of aqueous sodium hydroxide (20%). After stirring for 6 hr. the solution was poured into water and was allowed to stand overnight at room temperature. The precipitate was collected (0.78 g., 43%). It crystallized from dilute dioxane in red needles, m.p. 259° dec.

Anal. Calcd. for $C_{21}H_{13}O_2N_7S_2$: N, 21.35. Found: N, 21.43.

2-Benzothiazolyl-(2)-3-p-nitrophenyl-5-benzothiazolyl-(2)-tetrazolium-bromide (VIa). To the refluxing solution of formazan (V, 1.0 g.) in ethyl acetate (200 ml.) was added a solution of *N*-bromosuccinimide (0.8 g.) in ethyl acetate. After some time a yellow solution was obtained. It was allowed to remain overnight and the precipitate was collected (0.4 g.). The precipitate was refluxed with alcohol containing water (about 30%), and filtered. The filtrate was evaporated and the substance obtained (0.25 g.) was crystallized from alcohol-water to which was added a few drops of hydrobromic acid, in brownish yellow prisms, m.p. 212° dec.

Anal. Calcd. for $C_{21}H_{12}N_7O_2S_2Br$: Br, 14.86; N, 18.22. Found: Br, 15.16; N, 17.98.

2-Benzothiazolyl-(2)-3-p-nitrophenyl-5-benzothiazolyl-(2)-tetrazolium chloride (VI). The bromide (VIa) was converted to the chloride (VI) by treatment with silver chloride in exactly the same manner as described for the chloride (II).

The chloride crystallized from water containing a few drops of methanol and a few drops of hydrochloric acid in orange-yellow needles, m.p. 199–200° dec.

Anal. Calcd. for $C_{21}H_{12}O_2N_7S_2Cl$: Cl, 7.19; N, 19.85. Found: Cl, 7.01; N, 19.60.

Oxidation of V with isoamylnitrite (VII). Oxidation of (V, 0.1 g.) was carried out exactly in the same manner as that of I, with isoamylnitrite (8 drops) and glacial acetic acid (10 ml.); yield (0.09 g.). The product (VII) crystallized from glacial acetic acid in stout yellow prismatic needles m.p. 211° dec. This acetate salt contained a nitroso group. It was reduced to a formazan by color test.

Anal. Calcd. for $C_{21}H_{11}N_7S_2O_2 \cdot C_2H_3O_2 \cdot NO$: N, 20.51. Found: N, 20.62.

Compound (VIII). The tetrazolium salt (VII, 0.1 g.) was, suspended ln absolute alcohol (15 ml.) and a stream of dry hydrochloric acid was passed through it with external cooling. The substance slowly went into solution and reprecipitated on further passage of the gas as an orange precipitate. On concentration the orange color changed to pink, which also occurred on just standing. It crystallized from alcohol or acetone in pale pink needles, m.p. 196–197° dec. On recrystallization colorless crystals were obtained. This compound failed to yield a formazan on reduction. Analysis gave N, 25.73; 25.31.

p-Nitrophenylhydrazone of benzothiazole-2-aldehyde. This compound was prepared from equivalents by mixing alcoholic solutions of benzothiazole-2-aldehyde (20%) and *p*-nitrophenylhydrazine (10%). It crystallized from dioxane in orange-yellow needles, m.p. 256° dec.

Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: N, 18.79. Found: N, 18.79.

2-p-Nitrophenyl-3-(3,3′-dimethoxy-4-biphenylyl)-5-benzothiazolyl-(2)-formazan (IX), and 2,2′-di-p-nitrophenyl-3,3′-(3,3′-dimethoxy-4,4′-biphenylene)-5,5′-dibenzothiazolyl-(2)-diformazan (X). The *p*-nitrophenylhydrazone of benzothiazolyl 2-aldehyde (0.98 g.) was dissolved in tetrahydrofuran (80 ml.), and dioxane (20 ml.). The solution was cooled to −20 to −25°. *o*-Dianisidine hydrochloride (0.52 g.) was suspended in water (2 ml.), and conc. hydrochloric acid (0.5 ml.). It was tetrazotized with an aqueous solution of sodium nitrite (0.25 g.) at 0°. The tetrazotized solution was then added to the above solution, followed by the addition of 15 ml. of sodium hydroxide (20%). It was stirred for 6 hr., at room temperature. It was then poured into a large volume of water. The precipitate obtained was collected, washed with methanol (100 ml.), with hot water, and again with methanol. To separate mono and diformazans it was extracted with benzene in a Soxhlet apparatus for a week, and with methanol or ethyl acetate for 2 days. Very faint pink color stained the final extract. The precipitate in the thimble weighed (0.70 g.; 51%). It was very sparingly soluble in pyridine. It crystallized from pyridine in dark-colored small needles; m.p. 294° dec.

Anal. Calcd. for $C_{42}H_{30}O_6N_{12}S_2$: N, 19.58. Found: N, 19.00.

The benzene and ethyl acetate extracts were evaporated and the monoformazan (0.20 g.; 23%) crystallized from benzene in dark prisms, m.p. 233° dec.

Anal. Calcd. for $C_{28}H_{22}O_4N_6S$: N, 15.61. Found: N, 15.59.

2,2'-di-p-Nitrophenyl-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-5,5'-dibenzothiazolyl-(2)-ditetrazolium bromide (XI). The diformazan(X, 0.1 g.) was powdered and suspended in glacial acetic acid (15 ml.). *N*-Bromosuccinimide (0.25 g.) was added, and the suspension was heated to boiling. The heating was stopped as soon as the color of the solution changed to yellow. The solution was filtered and the filtrate was evaporated in the hood at room temperature. The residue was extracted with boiling water, filtered, and evaporated on a steam bath, after the addition of a few drops of aqueous hydrobromic acid. It crystallized from its solution in hot water after the addition of a few drops of hydrobromic acid in a pale yellow powder; yield (0.1 g.). The substance did not melt up to 250° but turned brown at 162°.

Anal. Calcd. for $C_{42}H_{28}N_{12}O_6S_2Br_2$: Br, 15.68; N, 16.47. Found: Br, 15.58; N, 16.79.

2,2'-di-p-Nitrophenyl-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-5,5'-dibenzothiazolyl-(2)-ditetrazolium chloride (XII). The bromide (XI; 0.1 g.) was suspended in distilled water (500 ml.). Freshly precipitated silver chloride (from 1 g. of silver nitrate) was added and the solution was refluxed on a metal bath for 8 hr. It was filtered, and the filtrate was evaporated on a steam bath; yield (0.08 g.). It dissolved in alcohol containing water and crystallized on concentration in yellow prismatic plates. The compound did not melt but became dark at 163–164°, after turning brown at 150°.

Anal. Calcd. for $C_{42}H_{28}N_{12}O_6S_2Cl_2 \cdot 6H_2O$: C, 48.50; H, 3.84; Cl, 6.83; N, 16.16. Found: C, 48.48; H, 3.86; Cl, 6.74; N, 15.90.

2-Benzothiazolyl-(2)-3-(3,3'-dimethoxy-4-biphenylyl)-5-phenyl-formazan (XIII), and 2-2'-dibenzothiazolyl-(2)-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-5,5'-diphenyldiformazan (XIV). A tetrazotized solution of *o*-dianisidine hydrochloride (0.32 g.) was added to a solution of benzaldehyde, 2-benzothiazolylhydrazone (0.50 g.) in tetrahydrofuran (30 ml.) at −20 to −25°. This was followed by the addition of aqueous potassium hydroxide (3.0 g. in water 15 ml.). After stirring for 4 hr. at room temperature, the reaction mixture was poured into water. The precipitate was filtered, washed with hot water, dried, and extracted with methanol in a Soxhlet apparatus, for 4 days. The residue (XIV) in the thimble weighed (0.18 g., 23%). It crystallized from pyridine in dark needles with a golden luster, m.p. 278° dec.

Anal. Calcd. for $C_{42}H_{32}O_2N_{10}S_2$: N, 18.13. Found: N, 18.00.

The methanolic extracts (XIII) were evaporated to dryness (0.30 g., 60%). This residue was further extracted with carbon tetrachloride in a Soxhlet. Carbon tetrachloride was distilled, and the residue was purified by precipitation from its benzene solution with petroleum ether in red needles, m.p. 156–58° dec.

Anal. Calcd. for $C_{28}H_{23}O_2N_5S$: N, 14.19. Found: N, 14.24.

2,2'-Dibenzothiazolyl-(2)-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-5,5'-diphenyl ditetrazolium bromide (XVa). The diformazan (XIV, 0.1 g.) was suspended in chloroform (60 ml.). *N*-Bromosuccinimide (0.4 g.) was added and the suspension was refluxed on a steambath for 2 to 3 hr. Refluxing was usually stopped when the color of the solution became yellow. The chloroform solution was concentrated to a small volume (10 to 15 ml.) and diluted with dry ether. The yellow precipitate obtained was collected (0.11 g.). The precipitate was dissolved in alcohol, treated with norite, and filtered. The precipitate obtained on dilution with dry ether was collected and extracted with boiling distilled water. The clear yellow filtrate was evaporated after the addition of a drop of aqueous hydrobromic acid. It was purified by crystallization in yellow prismatic plates from its aqueous solution on addition of aqueous hydrobromic acid. The substance was dried in a vacuum desiccator over phosphorus pentoxide; m.p. 148° dec., yield (0.085 g.).

Anal. Calcd. for $C_{42}H_{30}N_{10}S_2O_2Br \cdot H_2O$: C, 53.17; H, 3.37; Br, 16.86; N, 14.77. Found: C, 53.15; H, 3.54; Br, 17.26; N, 14.90.

2,2'-Dibenzothiazolyl-(2)-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)-5,5'-diphenyl ditetrazolium chloride (XV). The bromide (XVa) was converted to the chloride in exactly the same manner as described for the preparation of XII. For crystallization, the compound was dissolved in alcohol containing a little water, concentrated to a small volume after the addition of a drop of hydrochloric acid, and cooled. It crystallized in golden yellow plates, m.p. 129–130° dec.

Anal. Calcd. for $C_{42}H_{30}N_{10}S_2O_2Cl \cdot H_2O$: C, 58.66; H, 3.72; N, 16.29. Found: C, 58.97; H, 3.85; N, 16.60.

Compound XVI. The diformazan (XIV; 0.1 g.) was oxidized with glacial acetic acid (4 ml.) and isoamylnitrite (5 drops) on the steambath. After most of the diformazan had dissolved, the yellow solution was filtered and diluted with ether. The precipitate (0.06 g.) was purified by repeated precipitation from its methanolic solution with ether, m.p. 167° dec. This yellow powder was poorly soluble in water, contained two nitroso groups, and was reducible to a formazan by color test.

Anal. Calcd. for $C_{42}H_{30}N_{10}O_2S_2 \cdot 2\ C_2H_3O_2 \cdot 2\ NO$; N, 17.72. Found: N, 17.70.

Histochemical Experiments. The tetrazolium salts were dissolved in water and diluted with standard media for succinic dehydrogenase[6] and DPN diaphorase[6] to a final concentration of 0.1 mg./ml. Frozen sections of liver, kidney, and stomach of the rat were examined. Chelation was demonstrated by including Co^{2+} in the incubation media at a concentration of $10^{-3}M$.

Reduction in the DPN diaphorase and succinic dehydrogenase systems occurred almost instantaneously with salt II and very rapidly with salt VI. This is consistent with their high position in Table I. Chelation with cobaltous ions to an almost black product in intracellular organelles occurred with II, but extensive crystallization occurred soon thereafter on storage, obscuring intracellular detail. Chelation was poor with VI and absent with XII. Nevertheless, XII gave intra-mitochondrial diformazan deposits similar to Nitro-BT, even though this diformazan was not as substantive for protein as the diformazan from Nitro-BT and was extractable from tissue sections with ethanol. This would indicate that the striking substantive properties exhibited by the diformazan of Nitro-BT is easily lost on structural changes such as introducing *ortho* or *para* iodo groups into the C-5 phenyl ring (7) or a benzothiazolyl -(2) group into the C-5 position. These experiments indicate that the benzothiazolyl -(2) group in the C-5 position not only does not favor chelation as it does in the N-3 position, but appears to inhibit chelation of a second benzothiazolyl -(2) group in the N-3 position. Salt II appears to be the most promising member of the present series for histochemistry by virtue of its readiness to accept electrons from the dehydrogenase systems and its readiness to chelate. Further exploration of the results of chelation with other metal ions would be worthwhile in order to eliminate the crystalline character of the chelate.

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