The Use of Triphenyltetrazolium Chloride for the Study of Respiration of Tissue Slices

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In 1948 Strauss, Cheronis and Straus suggested the use of triphenyltetrazolium chloride for the differentiation of cancer tissue from surrounding normal tissue. The authors indicated that tumor tissue would reduce the dye more readily than normal tissues (*2*). The use of triphenyltetrazolium chloride was also mentioned by Kun and Abood (*1*) in a study of the respiration of tissue homogenates. Our paper will describe a technique whereby the respiration of tissue slices may be measured with the aid of 2,3,5-triphenyltetrazolium chloride, and will present some results of these measurements.

The tissues which were studied include liver, kidney, spleen, diaphragm, small intestine, and spontaneous mammary carcinoma, all from CFW mice. With the exception of the diaphragm, tissues are sliced freehand, at a thickness of approximately 0.5 mm. The diaphragm was not sectioned, since it is thin enough to permit ready diffusion. All sections were run in duplicate so that reproducibility of the results could be evaluated.

The animal was usually killed rapidly by crushing the cervical cord, and sections of the organs were made within a few minutes of death. The tissue sections were placed in wide-mouthed test tubes (1.5 × 10 cm) and 5 ml of normal saline was added to remove blood and extraneous material. The solution was then decanted and 3 ml of the buffered (pH 7.2) 1% tetrazolium solution in distilled water was added. The tubes, placed in a rack in an incubator maintained at 37° C, were agitated gently by means of a mechanical shaker. After incubation the tubes were removed, the tetrazolium solution was decanted, and the red color of the reduced tetrazolium extracted with acetone. The extraction was a quantitative one accomplished by means of repeated washings with acetone. Each washing was added to a Klett colorimeter tube. Usually four washings of 2 ml, 1 ml, ½ ml, and ½ ml were sufficient to remove all the color from the tissue. The acetone in each colorimeter tube was then diluted to the 5-ml mark with additional acetone, and after thorough mixing, the intensity of the color was read in a Klett-Summerson photoelectric colorimeter, using the green No. 42 filter. The tissues were allowed to dry at room temperature, a procedure which was accomplished rapidly because of the previous acetone dehydration. The tissues were then weighed on an analytical balance and the color reading determined per mg of tissue. The color readings can also be converted to gamma of tetrazolium reduced, by the use of a standard dilution curve of reduced tetrazolium as indicated by Kun and Abood.

TABLE 1

REDUCTION OF TETRAZOLIUM BY DUPLICATED TISSUE SLICES (30-MIN INCUBATION)

Tissue	Colorimeter reading	Tissue weight in mg	Reading per mg
Liver, a	131	3.1	42.2
Liver, b	140	3.1	45.1
Kidney, a	88	1.6	55.0
Kidney, b	226	3.7	61.0
Tumor, a	120	6.7	17.8
Tumor, b	146	7.3	20.0

The values obtained by this technique were reproducible in duplicate slices as shown in Table 1. The mean values as well as the spread of values for the endogenous metabolism of the various tissues of CFW mice, with and without spontaneous mammary carcinoma, are shown in Table 2.

It will be noted that the values obtained are similar in tissues from animals with and without spontaneous breast carcinoma. The only apparent exception to this is in the case of the diaphragm after 30-min incubation, where the mean value obtained was lower in the presence of the tumor. However, after a 60-min incubation period the mean values were identical. The relative intensity of the endogenous metabolism as measured by this technique (with 1-hr incubation) is in the following order: kidney, liver, duodenum, diaphragm, tumor, spleen. It also appears significant that while the kidney, liver, and duodenum show increased reductions of the dye after 60-min incubation as compared with 30-min incubation, this was not the case with the tumor and spleen, wherein little change occurred, and with sections of diaphragm, where an actual decrease in values was observed. The exact significance of this observation is not clear at present and is being studied further.

TABLE 2

COLORIMETRIC ESTIMATION OF REDUCTION OF TRIPHENYLTETRAZOLIUM CHLORIDE BY TISSUE SLICES

Organ	30-min. incubation: No. animals	Lowest value	Highest value	Mean	60-min incubation: No. cases	Lowest value	Highest value	Mean
Female CFW mice with spontaneous mammary carcinoma								
Liver	10	33	53	40	7	47	82	65
Kidney	10	41	63	55	5	79	112	99
Diaphragm	5	19	43	33	4	20	37	29
Small intestines	2	43	50	46	4	39	76	56
Tumor	7	7	33	17	4	18	24	22
Spleen	4	11	41	22	13	8	32	18
Male and female CFW mice without tumor								
Liver	6	34	52	41	17	53	93	63
Kidney	6	44	71	52	12	83	141	91
Diaphragm	5	24	76	52	5	20	36	29
Small intestines	4	26	55	41	4	39	77	56
Spleen	2	9	18	15	7	10	32	16

This technique is also applicable to the investigation of the effects of enzyme inhibitors on tissue respiration.

Thus the addition of sodium azide to the tetrazolium solution resulted in depression of the reduction of the dye. Typical results are indicated in Table 3, which presents

TABLE 3

EFFECT OF NaN_3 ON REDUCTION OF TRIPHENYLTETRAZOLIUM CHLORIDE BY TISSUE SLICES

Tissue	R_0*	% Change 10^{-3}M	NaN_3† 10^{-6}M
Liver	44	−45	−11
Kidney	55	−36	− 2

* R_0 = Colorimeter reading/mg tissue, dry wt, 30-min incubation.

† Sodium azide, 0.5×10^{-3}M and 0.5×10^{-6}M added to 3 ml of 1% tetraphenyltetrazolium solution.

the mean values for tissues from six animals, the experiments having been done in duplicate.

It appears from the data presented that triphenyltetrazolium chloride may be used in a simple and reproducible manner to study the metabolism of tissue slices and the effects of enzyme inhibitors.

References

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Electrolytically Induced Reactions

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Recently two examples of a novel type of induced reaction have been observed in this laboratory. Both reactions are unique in being electrolytically induced. The first is the electrolytically induced air oxidation of trivalent arsenic, As^{III} in alkaline solution. It has been observed (*1*) that air oxidation of As^{III} in a solution 1*N* in sodium hydroxide is slight. But if electrolytic oxidation of the arsenic is carried on in the same solution, oxidation occurs up to 50% in excess of that corresponding to the current used. This excess oxidation is attributed to oxygen of the air, since it is entirely eliminated if the surrounding air is displaced with nitrogen. The electrolytic process, however, seems essential.

The second reaction is the electrolytically induced precipitation of iridium with rhodium. It was observed by MacNevin and Tuthill (*2*) that iridium could not be deposited from an ammonium chloride solution at cathode potentials as great as − 1.0 volt. But if rhodium is also present, then not only does the rhodium precipitate quantitatively at a cathode potential of − 0.3 volts, but the major part of the iridium does also.

These two reactions are considered to be examples of a new type of induced reaction and it is proposed to call them "electrolytically induced" reactions.

References

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The Isolation of a Metabolite of Pentobarbital[1]

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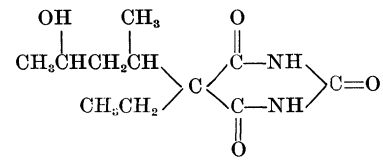
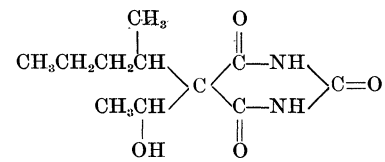
There is general agreement that pentobarbital is excreted only in trace amounts in the urine (*2, 6, 8, 9*), but up to the present no metabolic products of the drug have been reported. It has now been found possible to isolate a metabolite of pentobarbital from the urine of dogs after anesthetic doses of the drug. The compound melts at 209–210° C and has an elementary composition corresponding to pentobarbital with one additional oxygen atom ($C_{11}H_{18}O_4N_2$). In sodium hydroxide solution it has the characteristic ultraviolet absorption spectrum of the dialkylbarbituric acids (*5*). In the range of 225–290 mμ the absorption curve has exactly the same shape as that of pentobarbital, but the extinction is about 7% lower. This would imply a molecular weight of about 242 for the new barbiturate. Further evidence for the barbituric acid ring was obtained by the preparation of a di-*p*-nitrobenzyl derivative (*3*).

The presence of a hydroxyl group was demonstrated by the reaction of the metabolite with acetic anhydride. A new compound was formed which crystallized from aqueous ethanol in the form of blunt needles; mp 147–148° C.



(I)



(II)

It had the elementary composition of the corresponding acetate ($C_{13}H_{20}O_5N_2$). On treatment with sodium hypoiodite in dioxane solution the metabolite yielded iodoform. It would thus appear that the compound could be either I or II. Structure II would not seem likely, however, because such a compound would probably react with sodium hypoiodite to yield acetic acid, not iodoform.

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