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Action of Mercurial Antiseptics on Muscle Oxydase.

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While studying the effects of various chemicals and toxins on the activity of freshly prepared muscle oxydase, the authors made some interesting findings regarding the behavior of certain inorganic and organic mercurial antiseptics. A modification of the Thunberg method of determining the decolorization of a standard solution of methylthionine chloride, or methylene blue, in specially constructed glass vacuum tubes was employed. After considerable experimentation the following procedure was found to give the most satisfactory and surprisingly uniform results. Leg and abdominal muscles were carefully dissected from adult white rats, which had been killed by arteriotomy (or cutting of the vessels of the throat), and minced with sharp scissors in a glass mortar. To several grams of this muscle was added an equal number of cubic centimeters of physiological saline. The whole mass was then ground up with clean sand in a porcelain mortar for half an hour and then strained through fine linen. In this way a uniform suspension of minute particles of muscle tissue in muscle juice and saline was obtained. One cubic centimeter of such freshly prepared muscle suspension was introduced with a pipette into a Thunberg tube. Two cubic centimeters of methylene blue solution were then added to the muscle suspension in the tube. This indicator solution was made of 8 parts of methylene blue, 1:2,000, and 6 parts of 0.1 molar solution of acid potassium phosphate. With a Cenco-Hyvac vacuum pump the air was exhausted from the tube, which was then placed in a water bath at 38°C.; and the time required to completely decolorize the solution was carefully measured. The vacuum in the Thunberg tubes was perfectly maintained by carefully greasing the stopcock with a specially prepared lubricant containing a little gutta-percha. Such a vacuum being maintained, very accurate readings could be repeatedly obtained with any one specimen of muscle juice. Chemical compounds to be studied were respectively added to tubes containing muscle suspensions and allowed to act for a definite period of time, after which the Thunberg solution was introduced and the test was performed in the manner described.

The effect of the inorganic mercury salts, mercuric chloride, mercuric iodide and mercuric oxycyanide on muscle oxydase was compared with that of 3 organic mercurial antiseptics prepared in these laboratories; namely, Mercurochrome or dibrom oxymercury fluorescein, Merodicein or monohydroxy mercury di-iodo resorcinsulphonphthalein, and Flumerin or the disodium salt of hydroxy mercury fluorescein. The effect on enzymatic activity of different concentrations of these chemicals, to which suspensions were exposed for varying periods, was carefully investigated. Table I illustrates the average of findings obtained with these mercurials and also the results of control experiments made with alcohol (70%), phenol and Liquor cresolis compositus, U.S.P. In connection with the present work, interesting studies were made with solutions of Mercurochrome to which minute quantities of inorganic mercury (mercuric chloride) had been added. By the Thunberg method even slight adulterations of such Mercurochrome solutions with inorganic salts could be readily detected, as may be seen from the table.

TABLE I.

Drug Used	No. of Exp.	Concentration 1 part in	Time of Exposure min.	Time Required to Decolorize: Muscle Juice Without Drug min.	Time Required to Decolorize: Muscle Juice With Drug min.
Mercurochrome	10	250	30	78	78
"	10	125	5	74	75
"	10	"	10	74	79
"	10	"	15	74	80
"	10	100	6	53	63
Merodicein	10	500	30	78	78
"	10	250	30	31	35
Flumerin	5	500	30	78	68
Mercuric chloride	10	5,000	30	78	91
" "	10	2,000	15	78	killed
Mercuric iodide	10	6,000	15	78	88
" "	10	2,000	15	78	killed
Mercuric oxycyanide	5	10,000	15	78	killed
Mercurochrome	5	250	60	63	64
Mercurochrome plus bichloride	5	10,000	60	63	180
Mercurochrome	5	250	10	31	34
Mercurochrome plus bichloride	5	20,000	10	31	43
Alcohol	5	70%	30	78	125
Phenol	5	250	30	78	82
Liquor cresolis comp.	5	"	15	78	killed
Oxyquinoline sulphate	5	1,000	15	31	51

Such a procedure offers a means of detecting solutions of spurious or fraudulent Mercurochrome which occasionally appear on the market. The organic compounds were found to be much less toxic for the enzymes than the inorganic mercurials. This inhibitory action or depressant effect of the various mercurials for the muscle enzymes does not run parallel to their antiseptic activity but is rather an index of their toxicity. Thus, Mercurochrome solutions, 1 :250 to 1 :100, while quite sufficient to destroy all bacteria, did not inhibit the action of muscle oxydase to any great extent. On the other hand, solutions of mercuric bichloride (1 :10,000 to 1 :5,000) were markedly depressant for the enzymes and after longer exposures killed them.

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Response of Adrenalectomized Rats to Phloridzination.

GERALD EVANS. (Introduced by C. N. H. Long.)

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It was shown previously[1] that when fasted rats were kept for 24 hours at ½ atmosphere, glycogen equivalent to 34% of previously existing stores and not accounted for by decreases in other carbohydrate, was laid down. This phenomenon was found not to occur in adrenalectomized rats.

To test further the importance of the adrenals in the new formation of carbohydrate (a) intact rats, (b) adrenalectomized rats and (c) rats with bilaterally demedullated adrenals were given daily 50 mg. of phloridzin in olive oil subcutaneously, and the urinary glucose, non-protein nitrogen and ketones determined.

The success of the demedullation was checked subsequently by serial section; no accessory adrenals were found in these animals.

The results for all are given in Table I. To exclude moribund values those for animals which did not survive at least 36 hours beyond the period of observation were not used in averages given.

It will be seen that the excretion of sugar, nitrogen and ketones is much diminished in adrenalectomized rats. The excretory values (ketones excepted) for demedullated animals equalled or exceeded those of intact animals.

[1] *Am. J. Physiol.*, 1934, **110**, 273.