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Materials and Methods

One hundred and ten cases coming to the mortuary of Department of Forensic Medicine, Maulana Azad Medical College for medico legal autopsy, were included in the study. Cases showing signs of decomposition were excluded. A detailed history was taken as to the circumstances of death with special reference to any signs and symptoms suggestive of myocardial ischemia like pain chest, sweating, collapse and shock. A note was as to the time of onset of illness and to the eventual supervening of death, and also hospital records were procured wherever possible.

A complete autopsy was performed to examine all body cavities and organs; the heart was removed and examined. The weight of the heart was recorded, and then the coronaries and their branches were examined individually. This was done by using a sharp scalpel and cutting transverse sections of the coronaries and their main branches at 3 mm intervals. Arteries examined included the left main, the left circumflex, the anterior descending, the right main and the posterior descending. The coronaries were examined for the degree of stenosis, presence of thrombus, calcification or haemorrhage into an atheromatous plaque.

The atria were examined for any thrombi, or dilatation, the ventricles for any hypertrophy by measuring the thickness of their walls. The heart was then cut into slices about 10 mm thick by means of a long sharp knife from the apex upward till the bicuspid and tricuspid valves. The cut surfaces of the muscles and the interventricular septum were examined for any signs of ischaemia like loss of moist luster, mottling, mottling with yellow ten infarct, yellow tan softening, red-gray depressed, infarct borders, or scarring.

The heart slices were briefly washed in cold running water to remove clots and blood, but not allowed to soak or macerate as the enzyme reaction is purely a surface phenomenon and prolonged washing elutes the contents of the superficial cell thus giving a false reading. The slices were then placed in glass jars having a diameter slightly greater than the diameter of the heart slices, a separate jar being used for each heart slice.

All the jars were properly labeled as to the origin of the heart slices. The heart slices were then covered by a suitable incubating medium freshly prepared for monobasic and dibasic sodium phosphate solution and the staining dye, with the pH of the incubating medium adjusted to lie between 7.5 & 8.5. The level of the incubating medium was kept at about 1 to 2 cms above the upper margin of the heart slice. The jars were then put into a water bath and incubated at 37^{O} C for about half an hour, depending upon the rapidity of the appearance of the dye on the surface of the heart. The solutions were gently agitated at intervals of 2- minutes to discourage stagnation of the reagent and causing excessive deposition of the dye on the surface of the heart slices thereby producing

artifacts. The slices were turned over after 15 minutes to prevent prolonged contact of the tissue with the bottom of the jar. When the slices showed optimal staining, the jars were removed from the water bath. The incubating medium was poured off and the jars were filled with 10% formalin. This immediately inhibited the enzyme reaction and increased the contrast between the normal and infracted muscle. The tissues were also fixed permanently so that the heart slice was preserved. The slices were then compared for difference in staining if any in areas of suspected myocardial ischaemia.

Preparation of Incubating medium

Materials

1. Nitro B.T. (2,2'di-p-nitro phenyl-5,5' - diphenyl - 3,3' - (3,3' - dimethoxy - 4,4' - diphenylene) ditetrazolum chloride).
2. T.T.C. (Triphenyl Tetrazolium Choride)
3. N/10 NaOH solution
4. Monobasic and dibasic sodium phosphate.

Nitro B.T. Method

Stock solutions of Monobasic and dibasic phosphate were prepared in distilled water at 0.1 M concentration and kept separately. Stock solution of Nitro B.T. was prepared in distilled water to make up a concentration 5 mg/ml. At the time of the experiment the two phosphate solutions were mixed to the required quantity to give a pH of 7.5. To this the solution of Nitro-B.T. was added to give a concentration of 0.5 mg/ml. This incubating medium was prepared fresh because storing usually changes the pH. The pH of the incubating medium is more important than the concentration of the dye. It the pH in the final incubating solution is below 7.4, the reaction does not take place and there in no deposition of the day on the surface of the tissue, but if the pH is 9 or above, a non-specific deposition of the dye occurs on the surface, irrespective whether it is the normal or the infarcted muscle.

On incubation in this medium the normal myocardium with full enzyme activity showed a surface deposition of dark formazan and the infracted areas showed either a complete absence of the dye or very much reduced deposition.

T.T.C. Method

Stock solutions of mono basic and dibasic phosphate were prepared in distilled water at 0.1 M concentration and stored separately.

Stock solution of T.T.C was prepared in distilled water approximately as a 2% solution. At the time of incubation the phosphate solutions were mixed to give a pH of 8.0. Then an equal amount of the T.T.C. solution was added. The addition of the T.T.C. usually reduced the pH which was restored by adding drops of N/10 NaOH solution and using pH meter to check the restoration of pH.

On incubation in this medium the normal myocardium with full enzyme activity showed a surface deposition of bright red formazan but the infarcted areas showed either a complete absence of the dye or very much reduced deposition.

Principle of histochemical staining

The normal myocardium reacts with the buffered oxidation reduction reagent in solution and gets stained. The stained myocardium is reddish blue in color in case of Nitro B.T. and red in case of T.T.C. there is loss of endogenous substrates, coenzymes and dehydrogenases in the infracted tissue and therefore there is no reaction with the regents and hence remains unstained. On fixing with formalin the unstained tissue turns into light yellowish brown color and thereby increases the contrast between, normal and ischaemic myocardium.

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