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Review of Literature

Myocardial ischaemia in its various forms very commonly causes sudden death; and many medicolegal necropsies are carried out all over the world on these cases. But important gaps still remain in the knowledge of the structural pathology of affected coronary arteries. For this we must know, for example, where arterial stenosis most frequently occurs, how much of the artery is likely to be affected, how often several arteries are involved, and the distinctive pattern of the disease in different age-groups. With this objective in mind 75 hearts obtained at necropsy from men who had died suddenly were studied by Crawford, Dexter & Teare[1]. To facilitate comparison, 25 hearts were studied in each of the age groups 20-39, 40-49, and 50-59 years. The youngest group comprised men aged 24, 26, 28, 30, 32, and 33 years; 2 aged 25; 3 aged 37; 3 aged 38; 5 aged 36; and 6 aged 39.

In all 75 cases the pathological process which had impaired the blood-carrying capacity of the coronary arteries was atherosclerosis. In many cases fresh or organizing occlusive or mural thrombi were present, but these were always on a basis of advanced atherosclerosis. No other pathological basis for the arterial disease was found in any of these cases. The frequency of recent occlusion and of severe stenosis of one or more arteries was recorded. An occlusion was regarded as recent when unorganized fibrin or atheromatous debris could be identified occupying the former lumen. Stenosis was assessed at two levels: reduction of the lumen to 1/3 of normal, and reduction to ¼ of normal. Lesser degree of stenosis was disregarded. The differences between the degree of stenosis and the frequency of multiple arterial involvements in the different age groups were remarkably small, and definitely not significant.

Of 48 recent occlusion studied, 39 were due to plugging of the lumen by thrombus, consisting of fibrin with an admixture of blood-cells. The appearance of all was consistent with thrombus formation in situ, and there was no suggestion that embolism had played any part in the process. In the remaining 9 occluded arteries, the plug consisted of atheromatous debris, either alone (2 cases), or mixed with thrombus.

All these obstructions by debris were in severely atheromatous segments of artery, so occlusion had probably developed close to the point at which the fatty debris had entered lumen proximally, and travelled to the site of obstruction as an embolus cannot be excluded. Hemorrhages into the substance of the diseased intima were frequently seen, both in occluded and in patent arterial segments, but no intimal haemorrhage was large enough to the close off the lumen. Occlusion was found only in vessels reduced in caliber to one third or less of the original, and most occlusions were in vessels with Lumina between one third and one fifth of normal area. In this study lesion

are added together the differences are insignificant. The distribution of the old lesions within the arteries follows the same pattern as that of recent occlusions.

Only the presence of calcification in an artery was recorded, with no attempt at quantitative measurement. There was a steep rise with advancing age in the number of arteries affected. 8 cases in the under 40s had no calcification in any artery, but there was only 1 such case in the two older age groups. The total number of arteries affected rose from 35 to 75 vessels in the under 40s to 56 in the 50-59 age group. The anterior descending artery was the one most frequently calcified in all the age groups. The relationship of calcification to occlusion and stenosis was analyzed. The occluded and the severely stenosed arteries show approximately equal frequencies of calcification, but the less severely stenosed vessels were less often calcified. The effect of age was again evident.

In the absence of blood pressure readings the only indication as to the frequency of hypertension comes from the heart weights. The hearts had been divided into 4 groups, somewhat arbitrarily, assuming that those under 390 gm were normal, those of 390-420gm were probably normal, those of 430-480gm show the increased weight which may result from coronary-artery disease or from hypertension, while in those over 480gm the hypertrophy almost certainly results from hypertension. If this is accepted, then there were 2-3 hypertensive subjects in the youngest group, 4-7 in the 40-49 age groups, and 4-6 in the oldest group, giving a total of between 10 and 16 hypertensives in these 75 sudden death victims.

In these cases of sudden death infarcts were present in only a few hearts. Scars were found much more often in the older subjects, but the few infarcts were found principally in the younger groups. Infarcts were most often in the distribution of the right artery, and scars in the region of the left anterior descending vessel. A scar or infarct was, without exception, accounted for by occlusion or severe stenosis in the corresponding vessel; but these were many arterial lesions which had not resulted in corresponding myocardial damage.

In every case the crucial lesion in the coronary arteries was atherosclerosis, with or without recent thrombosis. This holds good both for the 9 who died with only a short segment of artery diseased, and for the more numerous cases in which confluent atherosclerosis affected 10-20 cm of artery. Only just over half the arterial occlusions involved in the left anterior descending artery the right artery was occluded in over a third of the cases, and the circumflex in a fifth. Apparently the reputation of the anterior descending artery as the site of a much higher proportion of thrombotic occlusions is not merited. The anterior descending artery is, however, almost constantly stenosed in these cases: its lumen was reduced to one third or less of normal in 73, and to one quarter or less in 70, of the 75 hearts.

Comparison between age-groups, which was one of the prime purposes of this study, reveals few significant differences. The prevalence of multiple occlusions and stenoses is the same throughout. Recent occlusion are somewhat more frequent in the younger subjects, old organized occlusions in the later age groups; and the degree of pre-occlusive stenosis is severe at all ages. The extent of confluent atherosclerosis is only slightly greater in the older groups but, as anticipated, more of their arteries were calcified. Though the heart increases in weight with age, the number of definitely hypertensive subjects is only slightly greater. Differences between the age groups are slight and quantitative, with a gradation from the youngest to the oldest. There is nothing to support the contention that under the age of 40 fatal coronary disease differs in any essential way from the disease in older subject.

Some observers attribute geographic, racial and sex differences in the incidence of myocardial infarction to differences in the severity of arterial atherosclerosis, others suggested that the modern epidemic of ischemic heart disease may be due to primarily to an increased tendency to thrombosis. Therefore, controversy still existed concerning the relationship of the occlusive episode and the underlying lesion in the arterial wall.

This issue had been investigated by Strong & McGill[2] by comparing arterial lesions in coronary death cases with those in age-sex-race matched accidental death cases. This study also describes the early development of mural lesions with age and provides a quantitative base line of lesions for comparison with other population groups. They studied coronary arteries dissected from the hearts in 548 necropsies on patients from 1 to 69 years of age. Grossly visible lesions were rare in the first 10 years of life, but increased rapidly in succeeding decades until their presence was almost universal in adults. In comparing the sex race groups, a trend emerged with the highest prevalence of lesions in the white male, lowest in the white female, and intermediate in the Negro male and Negro female. Fibrous plaques appeared in white males two decades before they occurred in either female group. Fibrous plaques were clearly more prevalent in white males than in any other group up to age 39. After age 40, fibrous plaques were present in most cases in all sex race groups. The coronary deaths on the average clearly had more coronary atherosclerosis than the case of accidental death; the difference was greatest in fibrous plaques and complicated lesions. However, there was great individual variation in each group as well as overlapping in ranges of the two groups. The overlapping between coronary and accidental deaths was expected in a population subject to a high risk of ischemic heart disease, since several of these accidental death victims would probably have developed clinical ischemic heart disease in subsequent years had they survived.

Myocardial infarction can occur in the presence of less extensive coronary atherosclerotic lesions, than the average for accidental death cases in the same age group, but it was clear that this occurrence was the exception rather than the rule in this series, (1 case out of 10).

In each age group total intimal involvement, fibrous plaques, and complicated lesions and (with one exception) calcified lesions were greater in the coronary deaths. The average extent of each type of lesion for the sex and race group by age was compared. White males had more total intimal surface involved from the second through the sixth decades. They also consistently had more fibrous plaques, complicated lesions, and calcified lesions in every group through the sixth decade.

The greatest quantitative difference between any 2 sex-race groups was the between male and females whites from ages 10 to 39. Here the males had approximately 3 times as great a proportion of the intimal surface involve as did the females, and there was an even greater difference in fibrous plaques and advanced lesions.

After age 40 white males still had more coronary lesion that white females, but the difference was not so great. Because of small number of female cases and wide individual variation in the degree of coronary lesions, not all of the age-specific male female differences in coronary lesions were significant.

The data from this study indicate a strong correlation between the arterial lesions of coronary atherosclerosis and ischemic heart disease in two ways: coronary atherosclerotic lesions are greater in ischemic heart disease deaths than in traumatic deaths, and sex and race specific

measures of coronary atherosclerotic lesions closely parallel death rates from atherosclerotic heart disease in the corresponding groups.

This correlation between coronary lesions and fatal ischemic heart disease suggests that the modern epidemic is based primary on atherosclerotic lesions rather than a terminal episode such as thrombosis, and that degree of coronary lesions in population is the determining factor in morbidity and mortality from ischemic heart disease. Not only do these observations support the hypothesis that ischemic heart disease was correlated with coronary artery lesions, but they also showed that the stage for the epidemic of morbidity and mortality in the 40's and 50's was set at least 20 years earlier, long before occurrence of the terminal occlusive episode.

The evidence suggested that coronary atherosclerosis directly predisposes to occlusion by thrombosis and thereby to ischemic heart disease. Patients dying from ischemic heart disease had a consistently higher mean extent of atherosclerotic lesions than accidental death cases or other natural death cases, but the ranges of the coronary and non coronary death cases overlapped. Ischemic heart disease did occur with coronary lesions less extensive than the means of accidental death cases, but this occurrence was rare.

The data support the hypothesis that coronary atherosclerosis was the most significant factor in determining the risk of ischemic heart disease in population. The important differences in coronary lesions developed very early in life and at least 20 years before the onset of manifest clinical disease. The search for etiologic factors responsible for coronary atherosclerotic lesions should therefore be concentrated on this earlier period of life.

Most investigators accepted the premise that the fatty streak was the earliest identifiable lesion of atherosclerosis, and that some fatty streaks progressed into fibrous plaques and other more serious atherosclerotic lesions. On the other hand, some evidence suggested that intimal fat deposition was an independent phenomenon unrelated to clinically significant atherosclerosis.

McGill[3] analyzed in overall pool of data extracted from grading of 23,207 sets of coronary arteries contributed from 14 countries to a central laboratory. The extent of fatty streaks in the coronary arteries and aortas of young person 10 to 39 years old from 19 geographic and ethnic groups were analyzed. Fatty streaks in the aorta increase rapidly in succeeding are groups during the third decade. Thereafter they end to diminish in extent or remain at approximately the same level. Fibrous plaques and other advanced lesions approaches similar degrees of extent 20 years or more lately. The extent of aortic fatty streaks in youth does not predict the extent of aortic raised lesions later in life on a group basis.

Fatty streaks in the coronary arteries increase more slowly in the second and third decades of life, and show no tendency to regress at any age. The mean extent of coronary artery fatty streaks in young persons appears related to the mean extent of raised lesions in the same population at middle age also coronary artery fatty steaks increase continuously in succeeding age group during the same period of life.

These data are consistent with hypothesis that advanced atherosclerotic lesions develop by progression and transformation of fatty streaks. However, the degree of which the transformation takes place appears to vary among arteries and among racial group.

Strong et al[4] studied a total of 2876 subjects, between 15 and 34 years old, black and white, men and women, who died of external causes and underwent autopsy to document the extent and

severity of atherosclerosis in adolescents and young adults in the United States. They found that intimal lesions appeared in the aortas and more than half of the right coronary arteries of the youngest age group 15-19 years and increased in prevalence and extent with age through the oldest age group 30-35 years. Fatty streaks were more extensive in black subjects that in white subjects, but raised lesions did not differ between blacks and white. Raised lesions in the aortas of women and men were similar, but raised lesions in the right coronary arteries of women were less that those of men. The prevalence of total lesions was lower in the right coronary artery than in the aorta, but the proportion of raised lesions among total lesions was higher in the right coronary artery than in the aorta.

Atherosclerosis begins in youth. Fatty streaks and clinically significant raised lesions increase rapidly in prevalence and extent during the 15 to 34 years age span. Primary prevention of atherosclerosis, as contrasted with primary revelation of clinically manifest atherosclerotic disease, must begin in childhood or adolescence.

Crea et al[5] reviewed the clinical angiographic and postmortem features of unstable angina and discussed its pathogenesis. The evidence of the assertion that coronary plaque inflammation may play a key role in the pathogenesis of unstable angina was examined and the assertion was found to be true. Finally the therapeutic implications of the involvement of inflammations in acute coronary syndromes were outlined.

The postmortem demonstration of myocardial infarction, especially in instances where a patient dies earlier than 8-12 hours, after showing the first signs and symptoms of ischaemia or collapses suddenly, had still remained a special challenge to the Forensic pathologist. Unequivocal gross anatomical changes of infarction do not become apparent for some 24 to 48 hours following the occlusion of a major coronary artery in man or 8 to 24 hours in experimental animals[7]. Furthermore even though minimal microscopic evidence of infarction can be recognized as early as 6 hours in man and 2 to 6 hours in experimental animals, these are very nonspecific and unreliable. In the absence of gross changes the involved area can be missed when random blocks are taken for histological examination.

With these handicaps in view, the attention of researchers had been directed towards finding a method of demonstration of myocardial infarction that could be applied easily to routine autopsy procedures and would also bring out the infarction in the first six hours after the appearance of signs and symptoms. During the search it was found that the earliest clinical sign other than the ECG tracings was the elevated levels of several enzymes into the blood following myocardial ischaemia. Being the earliest sign, it was regarded a frontier that would give the researchers enough headway if they could demonstrate that the elevated serum enzymes were actually released into the circulation from the infracted myocardium, and that the enzymes in the normal myocardium did not alter[9].

Once the path of investigation was determined, various methods were adopted to reach the goal. Mallory and associates studied the speed of healing of myocardial infarction[6]. They observed that no gross anatomical lesion could be defined before 48 hours with naked eye examination. They also found that the severity and duration of the occlusion was the main factor in determining the outcome, whether ischaemic necrosis followed by fibrosis or complete healing when the ischaemia was corrected by collateral circulation.

Wachstein and Meisel[7] tried to estimate the activity of succinic dehydrogenase in myocardial infarction and in induced myocardial necrosis. They found a drop in succinic dehydrogenase activity in the ischaemic myocardium in 6 to 8 hours.

Jennings et al[8] observed the effect on the Co-enzyme-I and carboxylase, after the ligation of coronary artery in dog. He produced ischaemia in a part of the myocardium by ligating the corresponding coronary artery; the ischaemic area was removed and studied for the two enzymes. It was found that the Co-enzyme I content of the affected heart muscle showed a consistent breakdown, varying in magnitude from 70 to 83% of the normal, within the first 12 hours. There was no appreciable change in the level of carboxylase.

Neoral and associates[9] applied the alterations of dehydrogenase activity to demonstrate myocardial infarction by macroscopic staining techniques. They divided their work in two phases; first they applied the method in the hearts of dogs that had their coronary arteries ligated to produce infarction. They used sodium tellurite as an oxidation-reduction indicator, and found that they could demonstrate macroscopically the infarcts that were 5 to 10 hours old. They subsequently applied the method in a small number of human hearts which were obtained during autopsy, and the subjects were clinically diagnosed as myocardial infarction. They found that they could demonstrate infarcted myocardium which was about 7 hours old. Reduction of sodium tellurite by malic dehydrogenase activity in normal myocardium resulted in a dark grey stain, while infarcted muscle with its decreased dehydrogenase activity remained unstained. However a drawback of this method was that it required incubation for 3-8 hours.

At about the same time Sandritter and Jestadt[10] applied the Triphenyl-tetrazolium chloride reaction (T.T.C.), to demonstrate the macroscopic delineation of myocardial ischaemia in guinea pigs. This oxidation-reduction indicator yielded a bright red formazan in the normal myocardium containing endogenous substrates and enzymes, whereas there was either little or no formazan formation at the site of the infarcted myocardium. They subsequently applied the method in a non-consecutive series of 112 autopsies. In 35 cases where fresh myocardial infarction was diagnosed clinically, but where the ordinary macroscopic evaluation did not give rise to a suspicion, fresh myocardial infarction was demonstrated by the dye. In 10 of the case, the fresh infarction demonstrated by the dye was found to be more extensive than suspected.

While giving their inferences they suggested that it was often possible to recognize infarcts at autopsy by this histochemical staining technique in patients who died as early as four hours after the onset of signs and symptoms.

Jennings and associates[11] studied the changes in the levels of succinic dehydrogenase (SDH), lactic dehydrogenase (LDH) and glutamic oxalacetic transaminase (GOT) in the infarcted myocardium as compared to that in the normal myocardium, in the first 24 hours after coronary artery ligation in dogs. Homogeneous myocardial infarction was produced in the posterior papillary muscle by ligation and the animals were sacrificed at regular intervals starting from 5 minutes to 24 hours. The samples of the infarcted muscles and controls from the left ventricle adjacent to the infarction were removed. The tissues were cut into small pieces and homogenized separately. The SDH, LDH and GOT levels were determined spectrophotometrically. The results that were obtained for all the three enzymes were plotted in separate graphs. All the three curves showed a similar shape, with an initial lag phase. This was followed by a period of accelerated loss till a level of 30-50% was

reached in 12-15 hours after the ligation. Little or no decrease was noticed for next 12 hours. The lag phase was 40-70 minutes for GOT, 2 hours for LDH and 4-5 hours for SDH.

Standjord and associates[12] studied the correlation between the enzymes released in the circulation following myocardial infarction and a subsequent fall of the enzyme levels in the affected myocardium. The enzymes that were included in the study were iso-citric dehydrogenase and lactic dehydrogenase.

Healthy mongrel dogs were selected and homogenous myocardial infarctions were produced by non invasive ligation. This was done to avoid the inevitable increase in the serum enzyme levels following thoracotomy. They observed the I.C.D. was elevated within 3 hours after ligation and returned to normal limits by 24 hours. The increase in the levels of LDH was not observed until six hours after the infarction; however the elevated activity was maintained as long as 44 hours. To make a comparison between the serum increase of the enzymes and their subsequent decrease in the infarcted myocardium, homogenates from the normal and infarcted muscles were prepared separately and the enzyme activity was estimated. The infarcted myocardium was found to contain correspondingly lower levels, matching with the increase in the serum levels at different time intervals compared with normal activity of ICD and LDH in the normal myocardium.

Klionsky[13] applied the histochemical method to study myocardial ischaemia and early infarct in rabbits. The coronaries in 14 rabbits were examined by injection techniques and grossly for the pattern and distribution of the vessels. The vessels were ligated in anaesthesia and then the animals were sacrificed at intervals from 1 minute to 120 hours. Sections were cut from each heart and treated with various techniques. A variety of histochemical methods were employed:- Prussian blue reaction for iron and the mureximide and alizarin red S techniques for calcium were used on tissues fixed in neutral buffered formalin, Von kossa's method for phosphates were used after alcohol fixation.

In a few animals stains for succinic dehydrogenase, TPN diaphorase, DPN diaphorase, sulfhydryl and disulphide groups were performed. In the ischaemic region the earliest and most striking change detected was the rapid loss of glycogen.

In tissues fixed in absolute alcohol and stained with PAS, a sharp zone of demarcation between normal and ischaemic muscle could be detected 5 minutes following ligation. As early as 2 hours after coronary ligation the infarct could be detected in haemotoxylin and eosin stained sections by cytoplasmic eosinophillia of damaged fibers and by perivascular infiltration with polymorphonucler leukocytes.

In 1960, Jennings and associates studied myocardial necrosis induced by temporary occlusion of a coronary artery. One hundred and fifteen dogs were used of which 45 survived for 24 or more hours after ligation, of these 36 were included in their study. Within 15 seconds of the occlusion, the posterolateral aspect of the heart become cyanotic and stopped contracting. The cyanosis persisted unchanged for the first hour after ligation and disappeared quickly with a flush, the moment the ligation was released. The previously cyanotic areas become red presumably by reactive hyperemia even in those dogs that were ligated only for 5 minutes. The hyperemia persisted for about 15 minutes and then became similar to the adjacent areas. Later when the animals were examined after 24 hours, they found marked necrosis related to the period of occlusion. Extensive pale grey subendocardial infarcts were observed in all the dogs occluded for 40 minutes or more. These infarcts were in marked contrast to those noted in the noted in the animals given shorter periods of

ischaemia, where the infarcts, though still sub-endocardial were much more focal. 22 minutes was the shortest time period that was followed by development of necrosis. No dog that survived the release after a period of 25 minutes of ischaemic injury was free of necrosis. Some capillary haemorrhage in the posterior papillary and elsewhere in the myocardium was common among the dogs that were subjected to 50-60 minute periods of ischaemia.

Graves and associates[14] studied the effects of myocardial infarction on the myocardial enzymes like glutamic oxaloacetic transaminase and lactic dehydrogenase. Hearts were obtained from routine autopsies and samples were taken from six regions of each heart, i.e. (1) Apex (2) Septum (3) Anterior wall of left ventricle (4) Lateral wall of left ventricle (5) Posterior wall of left ventricle and (6) Right ventricle. Histological sections were prepared from tissues immediately adjacent to the fragments taken for enzymatic studies. Determination of glutamic oxaloacetic transaminase and lactic dehydrogenenase was made on 40 hearts. These were divided into into four groups.

Group I (control) : This group consisted of 14 hearts in which there was no clinical or anatomical evidence of myocardial infarction. Group II: This group consisted of 14 cases with both clinical and anatomical evidence of fresh myocardial infarction. Group III: This group consisted of 8 cases where the clinical and anatomical evidence showed either healing or healed myocardial infarction. Group IV: This group consisted of 3 cases which showed no histological evidence of myocardial infarction or necrosis, fatty or fibrous replacement or any other histological change.

It was found that in group I there was minimal variation of transaminase levels in the six sites of a single heart. Groups II & III showed significant decrease in the concentration of transaminase in certain areas as compared to other areas of the same heart. Group IV did not show any difference in the concentration of the enzymes in various parts. It was also observed that in 7 cases where there was significant difference in concentration of transaminase in different areas of the heart, there was no appreciable decrease in the lactic dehydrogenase content in the same areas.

Nachlas and Shnikta[15] applied the Nitro B.T. method for macroscopic identification of early myocardial infarction both in experimental animals and human hearts during autopsy. In 40 dogs the anterior descending branch of the left coronary artery was ligated within 1.5 cms from its origin and just distal to the exit of the first major branch. In six dogs the ligatures was loosely applied at first and 2-3 weeks later the ligatures were tightened by traction, these animals were sacrificed 6 hours after the ligation of the coronary arteries. The hearts were examined 1 to 120 hours after coronary ligation, the animals being sacrificed at different intervals during the period. Sections were made through both ventricles having a thickness of 5 mm each. Early in the course of this investigation, the heart slices were placed in various substrate media employed for histochemical demonstration of succinic dehydrogenase, DPN diaphorase lactic dehydrogenase, malic dehydrogenase, TPN diaphorase, lactic dehydrogenase, malic dehydrogenase TPN diaphorase and cytochrome oxidase. The Sodium Tellurite and Triphenyl-Tetrazolium chloride methods were also compared with those of Nitro B.T. method. It became apparent that of the dehydrogenase reactions, no one method in particular was strikingly superior to the other for the gross demonstration of myocardial infarcts of 24 hours duration. These preliminary experiments also indicated that greater contrast between the normal and injured myocardium could be obtained in early infarcts by making conditions for the histochemical reactions suboptimal.

The incubation solution which was found to be best suited for the macroscopic identification of myocardial infarction 2 to 24 hours after coronary ligation consisted of Sorensen’s phosphate

buffer (0.1 M) PH 7.4 and 0.5 mg per ml of Nitro B.T. For convenience stock solutions consisting of phosphate buffer at 1 M concentration and Nitro B.T. at 5 mg per ml were kept. When ready for use, the incubation solution was prepared by mixing 1 part buffer 1 part Nitro B.T. and 8 parts of distilled water. The slices of dog hearts were rinsed briefly in running water to remove traces of blood and tissue juices from the cut surfaces and then were placed in the buffered Nitro B.T. solution for 30 minute incubation at 37^0C in a water bath. Normal myocardium stained dark blue within 15 minutes, while the infarcts remained unstained or stained faintly.

In order to determine which anaerobic dehydrogenase of heart muscle was mainly responsible for the production of the formazan pigment form Nitro B.T., experiments were carried out in which various substrates, succinate, lactate, malate, isocitrate, DPN, TPN and glucose and dehydrogenase inhibitors, malonate, oxalocetate, iodoacetate were added to the standard buffer Nitro B.T. solution. The rate of staining of normal and infarcted muscle in comparison with similar controls incubated without a substrate was then noted. The effects of postmortem autolysis of normal myocardium were assessed by storing portions of canine and human hearts at room temperature and at 4^0C for periods up to 72 hours. Freshly cut slices were then incubated in the standard buffered Nitro B.T. solution with or without the added substrates. It was recognized from these experiments that it would be advantageous to add succinate to the standard buffered Nitro B.T. solution when dealing with human heart, obtained after a postmortem interval of more than 6 hours in order to compensate for loss of endogenous substrates from normal heart muscle. Storage for 48 hours at 4^0C, resulted in patchy and erratic staining with some areas showing the expected dark blue pigment and other areas being unstained. In several experiments the Sodium Tellurite and T.T.C. methods were also tried with aged specimens. The reactions with these less sensitive redox dyes were less dependable. Storage for 6 hours at 37^0C and 12 to 16 hours at room temperature (25^0C) produced effects comparable to those observed after 48 hours in the refrigerator.

In 15 dogs out of the 40, with periods of coronary artery occlusion varying from 1 to 4 hours before sacrifice, gross examination of the heart did not furnish any suspicion as to the presence of a myocardial infarction. However after the enzyme reaction had been performed, the infarct could be recognized in 12 of these 15 hearts.

The method was then applied to 21 human hearts from persons with clinical histories of atherosclerotic heart disease. Autopsies were performed 2 to 70 hours after death. Seven of the patients had died suddenly or within minutes of the onset of acute chest pain or dyspnea. Six patients with “acute myocardial infarction” had died 2,7,8,30,40, and 55 hours respectively after the onset of typical acute symptoms; in four of these cases the diagnosis was supported by ECG and laboratory findings. Six other patients had succumbed during convalescence, 1 to 4 weeks after myocardial infarction. Two cases of severe congestive heart failure with recent infarction were also included in the series. Of the 21 patients, 8 were known to have had one or more earlier clinical episodes of myocardial infarction followed by recovery. The results with human hearts obtained at autopsy closely paralleled those encountered in dogs sacrificed at various intervals after LAD occlusion making due allowances for the differences in postmortem intervals.

Of the 21 patients with clinical atherosclerotic heart disease all showed stenosis or occlusion of major branches of coronary arteries. Fresh thrombotic occlusions were found in 4 hearts. The hearts from patients who had died suddenly or within minutes of the onset of symptoms and from 2 patients with severe congestive heart failure, showed no decrease in staining with standard Nitro B.T. solution or gross or microscopic evidence of recent myocardial infarction. Similarly in the heart

of the patient who survived for 2 hours, there were no distinct bio-chemical or structural changes in the myocardium, apart from old interstitial fibrosis.

In one case in which the time interval between onset of symptoms and death was 8 hours, areas of congestion and slight discoloration suggested the sites of very recent infarction, but did not precisely define their boundaries. The histochemical reaction outlined a recent infarct in the anterior wall of the left ventricle and interventricular septum. In 3 cases in which myocardial infarction had taken place 30 to 55 hours before death and in 6 remaining cases with infarcts 1 to 4 weeks duration, areas of necrosis of muscle, frequently bounded by a narrow hemorrhagic zone were seen on gross examination.

Even though the presence or absence of healing infarcts could be established readily by gross inspection at autopsy, the Nitro B.T. reaction was still of value because it outlined large and small areas of necrosis precisely with a resolution of 0.5 mm, permitted the identification of tiny islands of viable muscle within apparent homogenous infarcts and facilitated the preparation of photographs of specimens by enhancing the color contrast between normal and necrotic muscle.

Fine and associates[16] conducted experiments on rats by producing myocardial infarction; an effort was made to make a comparative study of the different histochemical methods and other methods like conventional H & E staining and P.A.S. staining. The left coronary of the animals was ligated and the animals were sacrificed from 5 minutes onwards after the ligation, at regular intervals. The stains that were used were Nitro B.T. in a buffered PH of 7.1, H & E stain and P.A.S. stain. Of the 34 hearts examined, 26 showed evidence of myocardial damage by one or more of the methods used. The enzymatic methods showed myocardial damage within 5 minutes after the ligation. The H & E staining showed the changes in half an hour. P.A.S. positive diastase resistant material was noted at 1½ hour.

Consequently, the experiments were applied to human hearts obtained during autopsy. The method chosen was the enzymatic technique using Nitro B.T. Here the hearts of 11 patients with a clinical diagnosis of myocardial infarction were taken. Each heart showed a severe narrowing or occlusion of a coronary artery without gross or microscopic confirmation of any myocardial infarction. The interval between the onset of symptoms and death was 0-4½ hours in 6 cases and was less than 12 hours in the remaining 5 cases. The staining was done by both conventional H & E stains and enzymatic methods for the sake of comparison. In none of the hearts did the H & E stain show evidence of myocardial infarction. Enzyme alteration was seen in 10 cases and the detectable loss of enzyme activity was from 1½ hour onwards. Glutaminanse was the earliest to start diminishing following by b-hydroxybutyerase and malate. Succinic dehydrogenase and cytochrome oxidase were the least affected.

Ramkisoon[17] applied the Nitro B.T. method of staining for macroscopic identification of early myocardial infarction of dehydrogenase alterations. Transverse slices of human hearts were obtained at autopsy from 31 patients with arteriosclerotic cardiovascular disease. These were obtained within 2 to 48 hours after death. The effect on the differential staining reaction by the loss of endogenous substrates and co-enzymes from early infarcts was assessed by adding succinate to the buffered Nitro B.T. solution. After the histochemical reaction employing Nitro B.T. had differentiated normal from necrotic myocardium, it was possible to correlate the gross findings with clinical and ECG evidence available on each patient.

The autopsy cases were divided into groups on the basis of clinical histories, Group I consisted of patients whose clinical histories were suggestive of recent myocardial infarction. There was ECG and laboratory evidence of myocardial infarction. Group II comprised of patients with known arteriosclerotic cardiovascular diseases, but without the usual clinical evidences of myocardial infarction. Group III included patients with other diseases which might be expected to produce secondary myocardial ischaemia during the terminal episode. The results that were obtained were : - Group I – in this group heart slices from 12 patients showed intense staining within 15 to 30 minutes in normal portions of the myocardium. Infracted myocardium on the other hand, showed little or no tissue staining. The earliest infarct showing loss of dehydrogenase activity was eight hours. Older lesions consisting of recent infarction and patchy interstitial fibrosis were also clearly demonstrated by this staining reaction. Group II – though this group of 15 patients did not show the usual signs and clinical symptoms of myocardial infarction however in each case there were areas of myocardial ischaemia or necrosis which delineated by little or no color development in the areas of infarction. Group III- heart slices from four patients from this group on histochemical reactions showed no staining or light staining areas of ischaemia, these subsequently disappeared with additional substrates and 30-45 minute incubation at 37^{O}C.

Brody and associates[18] used the Nitro B.T. method of staining for identification and delineation of myocardial infarction. The hearts of 35 patients suspected to have died of myocardial infarction were examined. The results that were obtained were divided into three groups. Group I – This group consisted of 21 patients where the myocardial infarction was demonstrated both by gross inspection and after Nitro B.T. staining Group II – consisted of 9 patients where the hearts did not show any myocardial infarction even after staining with Nitro B.T. In one case there was an unsuspected positive result. In 4 cases the fresh infarct, demonstrated after staining with Nitro B.T. was more extensive than suspected on gross inspection. In 17 cases the findings before and after staining showed conformity.

Jimmy and associates[19] embarked upon the study of the zone surrounding acute myocardial infarction. This was done to see its morphology as detected by dehydrogenase staining like Nitro B.T., and compared with those seen by conventional H & E stains. The myocardial infarction was experimentally produced in mongrel dogs. The anterior descending branch of the left coronary was doubly ligated and sectioned 3 cms below the origin, producing an area of myocardial ischaemia in the antero-apical region of the heart. It was observed that in those sections, that were stained with H & E nonspecific changes consisting of interstitial edema, the migration of a few neutrophills into congested vessels and hyperemia of a few small venules and capillaries could be detected in the area of suspected myocardial ischaemia as early as 1 to 2 hours after ligation. However, no definite ischaemic changes were discernible with H & E stain until approximately 12 hours after ligation. With application of Nitro B.T. method for showing dehydrogenase activity, pathological changes in tissues were observed much earlier than conventional techniques. Following the induction of ischaemia the enzyme stained myocardium could be separated into three zones. The central zone showed the area of disruption of the normal myocardial architecture and complete absence of enzyme activity. The second zone consisted of large dots' showing cell swelling only. The third zone consisted of fine dots' which showed the original architecture of myocardium.

McVie[20] applied the enzymatic method of staining to 65 hearts to find out the sensitivity and specificity of the method. These comprised of 29 patients who had died suddenly or within 12 hours of the onset of symptoms suggesting myocardial infarction, 20 patients were those that had died

due to clinically proven myocardial infarction and 16 patients were those who had died of some other causes and had non-infarcted hearts. Areas of infarction not visible to the naked eye were revealed in a transventricular section of the heart using enzymatic stains. Positive results (absence of staining) were obtained in all infarcts older than 12 hours. 10 cases out 20 which had intervals shorter than 12 hours and showed unapparent infarction on gross inspection – gave a conclusive result after staining. The earliest unapparent infarct revealed by the stain was 3½ hours old. The mapping of the infarct was beautifully portrayed by the above technique and provided simple correlation with the supply of an occluded coronary vessel.

Andersen & Hansen[21] made an effort to assess the value of Nitro B.T. in the diagnosis of myocardial infarction. The method was the same as that used by Nachlas et. al. The study was conducted on 100 hearts obtained at autopsy. Of these 40 cases showed fresh myocardial infarction in the left ventricle and three of these also in the right ventricle – in 15 of these cases the infarction was seen only after the Nitro B.T. test had been performed. 13 of the 40 cases were women with an average age of 77 years and 27 were men with an average age of 69 years. The location and extension of the myocardial infarction in the 15 cases in which infarction was revealed only after the Nitro B.T. test was very bizarre and affected almost any portion of the ventricular wall or the papillary muscle. None of the cases showed any coronary Thrombosis but one had sub-intimal haemorrhage in both the coronary arteries. There were two transmural infarctions. Four total infarctions and seven localized infarctions of which three were those of papillary muscles and one complicating sub-endocardial infarction. In one case there was a 6 mm fresh infarction located centrally in the marginal part of the wall of the left ventricle. The study revealed that Nitro B.T. was definitely more superior to the conventional histological methods.

Hansen[22] described an autopsy protocol that was meant specifically for the study of hearts from patients succumbing to heart diseases. The protocol was employed in a consecutive series of 63 patients with ischaemic heart disease. In the method adopted, the heart was removed as a whole, weight and measurements were taken and any visible changes in the pericardium were recorded. The coronaries were sectioned transversely at 6-8 mm intervals and they were inspected for any gross lesions of narrowing. The luminal narrowing of the coronaries was recorded in four groups. Groups i.e. mild stenosis, where about 25% of the coronary lumen was occluded by atherosclerosis. Group II i.e. moderate stenosis, where about 50% of the coronary lumen was occluded by atherosclerosis. Group III i.e. Severe stenosis, where about 75% of the coronary lumen was occluded by atherosclerosis.

Group IV i.e. Complete occlusion of the lumen by atherosclerosis. The ventricular myocardium was transversely sliced into 1 cm slices, washed in running water and inspected for any sings of acute myocardial infarction, myocardial fibrosis, aneurisms, ruptures, changes in the myocardium and mural thrombi. The Nitro B.T. test was performed on the slices and the results obtained were recorded in similar schematic diagrams. The quantization of acute myocardial infarction was done by point counting and to avoid shrinkage this was completed within 2 days of fixation in formalin. Each of the Nitro B.T. tested slice was covered by a transparent cellulose film on which regular net quadrants measuring 2 x 2 mm were drawn. The total area of the left ventricular mass including the interventricular septum was counted first and accordingly infarction was counted next and made record of. The results were recorded in the percentage of the total area that was involved in myocardial infarction. In case of the right ventricle, the quantization of the area involved in myocardial infarction was performed on the free right ventricular wall.

The influence of autolysis on the demonstration of myocardial infarction by histochemical methods has attracted the attention of several workers. Buss[23] found it impossible to apply the method in animals after 8-12 hours of death. Nachlas and Shnikta[15] did not find the method applicable after 8 hours if the experimental animals had been kept between 25-37^{O}C, at 4^{O}C however it was still applicable after 48 hours.

Morales and Fine mentioned that they used the method as late as 36 hours after death and Ramkisoon upto 48 hours after death. Andersen and Hansen were able to apply the method 72 hours after death, the bodies having been kept 4^{O}C. The macroscopic staining reactions were of the same intensity as of those examinations made within the first 24 hours after death.

Markil Gregersen[23] conducted a survey on one hundred and fifty cases of sudden unexpected death that could be attributed to acute myocardial lesions often secondary to coronary disease. He reports that for a detailed examination of the myocardium it is necessary to cut the heart in transverse sections up to at least the middle of the ventricles. This is an excellent method for showing the subendocardial infractions. The early infarction could be seen as slight changes in color and later as the well known yellow necrotic areas.

As a supplement the nitroblue tetrazolium test has been used and it had not only shown several infarctions which were invisible in unstained slices but also had often disclosed a greater extension of an infarction than that could be seen in untreated slices.

In this survey subendocardial infarction occurred frequently in higher proportion than has been observed in surveys from pathology departments. It is the author's hypothesis that all infarcts start in the subendocardial zone. This was supported by the fact that the first changes after a total occlusion of a coronary artery are seen in the subendocardial zone. In the fully developed transmural infarct the changes are most pronounced in the subendocardial zone. The basis for this is the special blood supply. The subendocardial zone was supplied with blood from long straight arteries which branch in the subendocardial. It has been said that the subendocardial exists event normally on the brink of hypoxia and there is some evidence in the literature that subendocardial infarcts can lead secondarily to thrombosis at a point of coronary narrowing and the evolution of transmural infarction. The conclusion of this survey was that the subendocardial zone should be studied very carefully in order to detect early myocardial changes.

Varga et al[24] studied the analysis of K/Na ion ratio of myocardium for the postmortem determination of the early myocardial infarction. The ion content of heart tissue was measured with flame spectrometer after the decomposition of myocardium by Lumatom tissue solublizer. The ion contents and their ratio correlate well with the electrocardiogram, the clinical data, the autopsy and histological findings. In some cases the change of the K/Na ion ratio was the only alteration that suggested an early stage of myocardial infarction. The Lumatom tissue solublizer can be very useful in determination of trace elements from any organic samples.

Luna et al[25] studied 144 pericardial fluids, from the same number of judicial corpses, and proposed the measurement of CK for the postmortem diagnosis of myocardial infarction, since it is a reliable, exact and easy technique available to an ordinary laboratory. Providing that contaminated samples be disregarded, the fluids are centrifuged early, and the cadavers be under 48 hours old, the study of CK enzymatic levels in the pericardial fluid allows the postmortem differentiation of myocardial infarction form other causes of death.

Saukko and Knight[26] studied the diagnostic significance of the eosin-fluorescence method was evaluated and compared with the enzyme histochemical beta-hydroxybutrate-dehydrogenase method and the degree of hyperchromasia in 568 samples from 24 beta HBDH-negative and beta-HBDH positive sudden cardiac deaths (SCH) and 23 non-cardiac deaths as controls. The effect of autolysis was investigated separately on isolated human hearts at +4^{O}C and + 22^{O}C. All samples were examined without any knowledge of clinical or autopsy data. Norma live-green fluorescence was observed in only five individuals of the control group and two in the beta-HBDH-negative one.

The frequency of the yellow-fluorescence and hyperchromasia increased from the control subjects towards the beta HBDH-positive ones. The difference in frequency distribution of fluorescence between groups was statistically highly significant (P <0.001). The change in fluorescence did not correlate with the loss of beta HBDH-reaction of the distribution pattern of hyperchromasia. Postmortem autolysis did not change the fluorescence significantly but the 78% wrong positive subjects in the control group render this method too sensitive and unreliable for medicolegal purposes.

Lachica E et al[27] studied the comparison of morphological, histochemical and biochemical methods applied to the detection of myocardial infarction in 150 medico-legal autopsies performed at the institute of Forensic pathology in Copenhagen. The study also included and NBT test of cardiac cross-sections, light microscopy and fluorescence microscopy of acridine orange-stained specimens from different sites of the cardiac musculature. Specimens of myocardium from the same four sites and pericardial fluid were analyzed biochemically at the Institute of Legal Medicine in Granada.

The K/Na ratio was determined in the myocardial tissue and total creatine phosphokinase activity, creatine phosphokinase isoenzymes (MM, MB and BB) and myoglobin were assayed in pericardial fluid. When the results from Copenhagen and Granada were compared, there was absolute inconclusive. After studying the circumstances of death, the number of discrepancies was reduced to 20, so that concordance was reached in 86% of all cases. The results show that the combination of different methods leads to a diagnosis of myocardial infarction in far more cases than with morphological or biochemical methods alone.

Rodrigues et al[28] studied 30 hearts from autopsies of suspected myocardial ischemia. They tried to evaluate the efficiency of the following methods, TTC test, histological examination of Haematoxyline and eosin section, fluorescent microscopy with Acridine orange and eosin auto fluorescence on this series of thirty patients. They conclude that acridine orange fluorescence method is the best method in diagnosing early cases of myocardial ischaemia when compared with TTC staining, H&E staining and eosin auto-fluorescence.

Lachica et al[29] had performed a study on 74 medicolegal autopsy case for the purpose of comparing the reliability of four different techniques (Haematoxyline-eosin stain, acridine orange method, formazan test and K/Na ratio) used for the postmortem diagnosis of myocardial infarction. Although the results showed that the formazan test and K/Na ratio give a good reliability to rule out a myocardial infarction, the most specific technique for the diagnosis of infarct is the Nitro-BT test.

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