Experimental Coronary Occlusion in Dogs and Its Effect Upon the Cardiac Glycogen Fractions

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A chemical quantitative analysis of the acid-soluble and acid-insoluble fractions of myocardial glycogen following an episode of acute infarction is presented. Both glycogen forms are depleted from the more seriously occluded area. The labile fraction similarly is decreased in the ischemic zone surrounding the infarct while the acid-insoluble moiety maintains a normal level.

The influence of a major occluded coronary artery upon myocardial glycogen seems to be associated very closely with oxygen availability and it has been shown that the glycogen content of an organ is usually directly proportional to the supply of oxygen. It has also been clearly shown that glycogen is depleted from the zone of infarction but some disagreement exists concerning glycogen accumulation at the borders of the infarct.

We were interested in the possible reactions of the acid-soluble (labile, acid-extractable glycoglycogen, free) and acid-insoluble (stable, desmoglycogen, bound, residual) fractions of glycogen, as well as endeavoring to reaffirm previously reported total glycogen changes, during ischemia after coronary occlusion. Secondly, it would be of interest to sample serially from the infarcted area to the normal area to determine if a "border zone" actually did exist and glycogen was significantly different from that of an infarcted area and a normal area.

One question still unresolved is whether or not glycogen may be metabolically active in more than one form. Presently we share the opinion of current investigators that the acid-soluble and acid-insoluble glycogen forms are functionally separate components. Meyer and Lourau have recently isolated and purified both moieties which adds considerable credence to the supposition that each form is a separate entity.

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Materials and Methods

Twenty-five adult dogs were anesthetized with sodium pentobarbital (32.5 mg./Kg, i.v.) and the anterior descending branch of the left coronary artery was ligated 3 cm. below its origin. No attempt was made to exclude the venae comitantes. After operation the chest was closed, the animals breathed naturally and heart activity was observed from electrocardiographic recordings. Typical anterior coronary occlusion records were obtained in most cases and have been reported extensively in the literature.

Three and one-half to 4 hours later the heart was removed and sampled for glycogen analysis. In 11 animals two pieces of ventricle were removed from each of 3 areas designated as: infarct, immediately below the ligated artery; border, approximately 1 cm. below and lateral to the tie; normal, above and lateral to the ligature. One piece of tissue from each zone or area was extracted with hot alkali and analyzed for total glycogen. The second tissue sample was immediately quick-frozen in dry ice and analyzed for acid-soluble glycogen. The difference represented the acid-insoluble glycogen fraction. In 14 hearts a strip of tissue was excised in a lateral-superior direction beginning 1 cm. below the ligature. It was divided into 6 equal parts which were labeled zones A (directly under the ligature and on the artery) through F (normal area). The acid-soluble and the acid-insoluble glycogen fractions were determined from each zone, the sum of the two being total glycogen available. No significant difference between zones C, D, E, and F was found. Consequently the glycogen values obtained from these zones were pooled and this is referred to as the normal zone. Zone A glycogen values were statistically similar to the originally labeled border area; zone B and the infarct area were likewise statistically similar.

Acid-soluble glycogen was determined by the procedure of Bloom, Lewis, Schumpert and Shen. The acid-insoluble glycogen fraction was determined by digesting the residue, obtained by centrifugation of the soluble fraction, in hot 30 per cent KOH. Both glycogen forms were subsequently hydrolyzed in sulfuric acid and quantitatively determined by the anthrone method as reported by Seifter, Dayton,
Novic and Muntwyler. Colorimetric determinations were made with a Beckman model B spectrophotometer.

**Results and Discussion**

The mean cardiac glycogen changes obtained from 25 animals are presented in table 1; statistical evaluations are given in table 2.

**Total Glycogen.** Analysis indicates that total cardiac glycogen decreased 47 per cent in the infarcted zone from the normal area in 3½ to 4 hours. For a short, but severe, period of infarction this figure agrees very well with those of Hermann and Decherd and Tennant, Grayzel, Sutherland and Stringer. There was observed to be a definite border area between the zone of infarction and the normal zone. Total glycogen decreased 23 per cent in the border zone with respect to the normal area but is 31 per cent greater than the infarcted area. The values presented for each zone indicate a high statistical significant difference (table 2). A previous series of experiments undertaken by this laboratory in which a complete internal pneumothorax was used to produce an acute anoxic situation in dogs resulted in a loss in left ventricular myocardial total glycogen of 26 per cent. This loss coincides very closely with the present decrease observed in the border ischemic area. The 21 per cent difference between experimental anoxic animals and the infarcted zone of coronary occluded dogs emphasizes the severity of a coronary attack in which a major artery is no longer patent.

**Acid-Soluble Glycogen.** At the infarct zone there is a very definite decrease in the two glycogen forms, however the relationship of each to the total indicates no relative per cent difference with respect to the normal zone. In normal cardiac tissue the acid-soluble component forms 69.5 per cent of the total; in the area of infarction the more labile form constitutes 69.8 per cent of the total available glycogen. This fraction decreases in the border area however and forms 53.6 per cent of the total. This represents a loss or change in this zone of approximately 16 per cent.

**Acid-Insoluble Glycogen.** This component has been referred to by many authors as the more stable fraction because it tends to fluctuate less in experimental situations than the more labile glycogen form. The acid-insoluble form at the zone of infarction shows a significant difference from either of the other two zones but its relationship to the more labile fraction is virtually the same percentage-wise as found in the normal zone. There is a slight increase above normal in this component in the border zone but the rise is not a significant one. It does form 45 per cent of the total in the latter zone, as compared with 31 per cent in the normal area, but this rise is primarily due to the decrease in the acid-soluble fraction resulting in a decreased total.

Quantitative chemical analysis of myocardial glycogen following experimental coronary occlusion has been done by a few investigators and in all cases there is the usual glycogen loss. Grayzel, Tennant and Stringer observed a depletion of glycogen ½ to 24 hours after the ligation of the left anterior descending coronary artery. In all cases the most significant and consistent decrease was found in the acid-soluble fraction.
in dogs. Himwich, Goldfarb and Nahum\textsuperscript{17} noted a glycogen decrease of 260 mg. per cent from the normal area of the left ventricle of some dogs. Herrmann and Decherd reported a 56 per cent decrease after 18 min. ligation, approximately 5 hours later the heart glycogen had decreased 87 per cent. Tennant and co-workers\textsuperscript{14} similarly found, 2, 4 and 8 hours after experimental ligation, losses of 39, 72 and 75 per cent respectively.

The present paper concerns the two moieties of glycogen. The tendency for the insoluble component to increase above its normal level in the less ischemic (border) zone suggests that in early and rapid acute anoxia there is a conversion of the labile fraction into the more stable moiety. This was suggested in the previously mentioned anoxic episodes. One of the two conditions where no significant difference was found was between the acid-soluble component of the infarct and border areas. Yet a very definite difference existed between the total glycogen of these zones. Apparently the two glycogen fractions tend to stabilize in the less ischemic or border area and stability of the acid-insoluble moiety is the first requisite. Fluctuations in the acid-soluble form are more easily tolerated. Utilization of radioactive glucose investigated by our laboratories and also reported upon by Dratz, Russell and Covey\textsuperscript{18} confirm the fact that a close metabolic correlation exists between the two glycogen forms.

SUMMARY

The effect of experimental coronary occlusion in dogs upon the myocardial glycogen fractions has been investigated. The acid-soluble and acid-insoluble glycogen fractions in the area of infarction are significantly less than in the unaffected area. The greatest loss occurred in the former component. Glycogen similarly is depleted in an ischemic or border area surrounding the infarct, but the decrease occurs only in the more labile fraction.

SUMMARIO IN INTERLINGUA

Esseva investigate le effecto de experimental occlusion coronari in canes super le fractiones de glycogeno myocardiae. In le region del infarcto le fractiones solubile in acido e non solubile in acido del glycogeno es significativemente minus que in le region non afficite. Le plus grande perdita occurriva in le prime de iste duo componentes. Similmente le glycogeno es deplete in un region ischemic o marginal circa le infarcto, sed iste depletion occurre solmente in le plus labile del fractiones.

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REFERENCES

13. Herrmann, G. and Decherd, G.: Creatine and
Staircase and Negative Staircase

In 1871, Bowditch made a basic discovery of great importance in cardiac adaptation. It consisted in the demonstration that the contractions of ventricular muscle are augmented during a succession of excitations applied at closely spaced intervals. He called the phenomenon Treppe, or, translated, staircase. This obviously showed that each contraction somehow alters the state of cardiac muscle to render it more responsive; in short, it corresponds to what is popularly designated "warming up."

Several explanations, not mutually incompatible, have been given of the ultimate processes concerned. These include the views (1) that some substance inducing a slightly hypodynamic state accumulates during rest which is released during activity; (2) that some waste material accumulates during contraction which first facilitates contraction and then induces fatigue; (3) that contraction creates a more favorable intracellular electrolyte environment for shortening of the myosin fibrils (Hadju, Szent-Gyorgyi, 1953; Moulin and Willbrandt, 1955) and (4) that some link in the still mysterious transmission of excitation from the membrane to the myosin fibrils is strengthened.

Suggestive evidence for the last hypothesis was provided by the confirmed observation that the enhancement of contraction is not accompanied by corresponding augmentation of transmembrane action potentials. (Hoffman, 1936; Trautwein and Dudel, 1954). Recent observations of Niedergerke support the conclusion that the staircase phenomenon appears to be a surface effect linked to changes in calcium ion concentration. This investigation has also brought forth evidence that under certain experimental conditions repetitive excitation can produce the reverse effect, namely, a progressive diminution in amplitude of cardiac muscle contractions. This has—perhaps inaptly—been called negative staircase. Since such reduction in size of contractions is accompanied by shortening of the action potential and loss of its plateau, negative staircase is attributed to a primary alteration of membrane excitation rather than, as in staircase, to an effect on the coupling between action potential and the contractile mechanism.

For details see R. Niedergerke, J. Physiol. 134: 569, 1956.
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