Effects of Liver Fractions on Myocardial Contractility

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Tyramine was crystallized and identified as one of the cardioactive materials in liver extract. Of many other compounds tested on the isolated papillary muscle of the cat, only methionine, ethionine, serotonin, heparin, testosterone, Menadione, and Dicoumarol showed activity.

Ever since the early experiments of Ringer, it has been known that isolated heart muscle contracts more forcefully and for a longer time in serum than in an artificial medium. Evidence implicating the liver as a source of such a humoral factor has been reviewed.1 In the search for the active compound(s) in liver, an impure extract was shown to mimic the effect of serum on myocardial contractility.1 More recently, a digitalis-like compound present in plasma, liver and adrenal medulla was crystallized and identified as palmitoyl lysolecithin,2 bearing out early indications that the cardioactive material in blood was associated with the lecithin fraction.3 There may well be other cardioactive substances in liver extracts. Twenty years ago evidence was obtained which suggested that tyramine was present in liver extracts.4 This paper describes the results of systematic studies of the effects of various liver fractions on mammalian myocardium and the identification of tyramine as one of the cardioactive compounds of liver extracts. The effects of many other compounds which occur in liver are also described.

METHODS AND MATERIALS

Measurement of myocardial activity was carried out on the isolated papillary muscle of the cat by a modification5 of the method first described by Cattell and Gold. The basic nutrient medium for the muscle was Krebs-Henseleit solution, altered to contain 1.5 mM of ionic calcium per L. If the muscle did not respond to the substance tested after 15 min. exposure, it was inferred that the substance was inactive. Phospholipid extracts were prepared by a method similar to that described by Milstone.6 The tissue was minced with a volume of acetone equal to twice the organ mass. The residue was suspended in five volumes of diethyl ether and allowed to stand overnight. The ether was taken off in vacuo and the residue was extracted with one volume of 95 per cent ethanol. Non-heat coagulable bovine liver fractions were obtained from Armour Laboratories. They have been labeled and described by the company as follows: Expar A, whole aqueous extract of liver; Expar B, 70 per cent alcohol-soluble portion of the aqueous extract of liver; Expar C, 70 per cent alcohol-insoluble portion of the aqueous extract of liver; Expar D, a subtraction of Expar B containing carbohydrate and choline; Expar E, a subfracion of Expar B containing all the essential amino acids, purines, pyrimidines, pantothentic acid, nicotinic acid and pyrodoxine; Expar F, a subfraction of Expar B containing all the essential amino acids, purines, pyrimidines, pantothentic acid, nicotinic acid and pyrodoxine; Expar L-1, a lipid fraction rich in glycerides and steroids; Expar L-2, lipid obtained from the hot aqueous extract of liver. Thromboplastin was obtained from the Difco Laboratories.

RESULTS

Liver Lipid Extracts. It may be seen (fig. 1) that the lecithin fraction of liver rapidly, markedly and reversibly increased contractile force of the isolated papillary muscle. Within 5 min., 200 mg./100 ml. increased contractile force by more than 70 per cent; doubling the concentration resulted in a 130 per cent increase in contractile force. With this higher dose, it required 25 min. for the maximum effect to be manifest. The effect of this higher dose was also reversible. Neither concentration affected the threshold of excitability.

Liver cephalin, brain lecithin and brain cephalin, in concentrations of 1 Gm./100 ml. had no effect on either excitability or contractility. Thromboplastin in concentrations as high...
Both qualitatively and quantitatively the effect of Expar L-2 resembled that of the lecithin fractions, while Expar L-1 depressed contractile force (fig. 2). Excitability was not significantly affected. Since this latter fraction contains lipid material, we tested several fat-soluble vitamins and steroids.

As shown in figure 3, testosterone, 0.5 mg./100 ml., depressed contractile force, but unlike Expar L-1, its effect was not completely reversible. Other steroids were tested, desoxycorticosterone glycoside (500 mg./100 ml.), cholic, dehydrocholic, desoxycholic, tamoeholic acids, each at 50 mg./100 ml. All were without effect.

Although suspensions of vitamin K₁ and vitamin K₁-oxide had no effect, menadione (10 mg./100 ml.), within 10 min. after its addition to the bath, induced a rise in the amplitude of contraction which was maximal at the end of 15 min.; subsequently, contractile force diminished to a level slightly greater than normal (fig. 4). Twenty minutes after the addition of menadione to the bath, the resting tension of the muscle began to increase, and this increase continued until the systolic and diastolic tension were equal (after 70 min.); the muscle contractions then stopped in systole.
636 EFFECTS OF LIVER FRACTIONS ON MYOCARDIAL CONTRACTILITY

FIG. 5. Effect of Dicoumarol on contractile force of the mammalian myocardium. Upper curve, systolic tension; lower curve, resting tension, in arbitrary units.

FIG. 6. Effects of some aqueous extracts of liver on myocardial contractility.

and maximal voltage (70 volts) could not induce contraction. This effect of menadione could not be abolished by washing the muscle. The threshold of excitability changed from 8.4 to 10.5 volts during the first 20 min. In view of the antagonistic relation between vitamin K and Dicoumarol with respect to blood clotting, it was of interest to test the effect of the latter on myocardial contractility and excitability.

In contrast to menadione, Dicoumarol (10 mg./100 ml.) stopped the muscle within 15 min. in diastole (fig. 5). Its effect was immediate in onset and could not be abolished by washing the muscle. Excitability was depressed from 9.8 to 10.5 volts.

Water-Soluble Liver Extracts. Figure 6 shows the positive inotropic effects of some of the aqueous extracts of liver. It can be seen that Expar E is the most potent of these fractions, almost 100 times as potent as the liver lecithin fraction. The activities of the fractions, in decreasing order of potencies, were E, B, F, D, A, C. Expar E in some muscles induced automaticity and often lowered the threshold of excitability. This was not a consistent property of any of the fractions, but none raised the threshold of excitability.

As mentioned, Expar E contains the essential amino acids, purines, pyrimidines and other compounds. For this reason, amino acids and other compounds were studied for their effects on myocardial contractility. Thresholds of excitability were measured only if contractility was effected.

Methionine (fig. 7), in a concentration of 0.005 M (75 mg./100 ml.), increased contractile force by 40 per cent. It required 10 to 15 min. for the maximal effect to be attained and this was reversible by washing. Doubling the concentration increased contractile force still further. There was no difference in potency between the d- and l-isomers. d,l-Ethionine and l-ethionine were as active as methionine. The effects of methionine and ethionine were additive and pretreatment of the muscle by ethionine did not affect the subsequent response of the muscle to methionine. Both compounds lowered the threshold of excitability slightly (e.g., from 9.1 to 8.4 volts).

5-Hydroxytryptamine creatinine sulfate increased contractility and excitability in concent-
Fig. 8. Effect of 5-hydroxytryptamine creatinine sulfate on myocardial contractility, showing desensitization of the muscle to previously effective low doses by high doses.

A concentration of 0.5 mg./100 ml. altered the threshold from 8.4 to 6.3 volts and 1.0 mg./100 ml. and higher levels induced automaticity. The concentrations refer to the creatinine sulfate salt of 5-hydroxytryptamine; the levels of free 5-hydroxytryptamine are about half (i.e., 43.5 per cent) those stated. Consistently, as shown in figure 8, the effects of 0.5 mg./100 ml. could no longer be elicited after the muscle had been exposed to higher doses.

Sodium heparinate in a concentration of either 0.1 or 1.0 mg./100 ml. reversibly enhanced contractile force (fig. 9). At a concentration of 100 mg./100 ml. contractility was reversibly depressed. None of these concentrations influenced excitability.

Other compounds were tested at a level of 0.01 M. The following substances were inactive: glycine, dl-alanine, dl-serine, dl-threonine, dl-valine, dl-leucine, dl-isoleucine, dl-norleucine, dl-lysine, dl-citrulline, l-arginine, l-histidine, dl-ornithine, l-proline, l-hydroxyproline, dl-aspartic acid, dl-glutamic acid, l-cysteine, l-cystine (3 × 10^{-4} M), dl-phenylalanine, l-tyrosine, l-tryptophane, glycyglycine, glycylalanine, alanylanine, alanylglycine, reduced glutathione, oxidized glutathione (0.005 M), glutamine, asparagine, sarcosine, betaine, and taurine. At 10 mg./100 ml., the following compounds were not active: creatinine, creatine, adenine, guanine, xanthine, hypoxanthine, thymine, uracil, cytosine, thiamine, riboflavin, nicotinamide, pyridoxine, calcium pantothenate, meso-inositol, folic acid, coenzyme 1 and α-tocopherol phosphate.

Crystallization and Identification of Positive Inotropic Substance from Expar E. Since none of the compounds tested had an activity similar to the liver fraction, fractionation of Expar E was undertaken. Preliminary experiments indicated that the active material was stable to boiling with 1 N HCl and 1 N NaOH. The material was subjected to the following procedure:

Amberlite XE-64 was washed in turn with concentrated HCl, H₂O, saturated NaOH, H₂O, and 7 L. of 0.01 M phosphate buffer at pH 6.6, and then equilibrated with 3 L. of the same buffer. Fifty grams of Expar E were dissolved in 2 L. of this buffer and placed on a 16 X 6 cm. column of the treated Amberlite XE-64. The nonadsorbed material was collected. One liter of 0.01 M phosphate buffer at pH 5.0 was run through the column, followed by 2 L. of 0.01 N HCl, 6 L. of 0.1 N HCl and 1 L. of 1.0 N HCl. The eluates were collected in 20 ml. portions neutralized and tested on the isolated papillary muscle.

After elution with 1300 ml. of 0.1 N HCl, positive inotropic activity became manifest, reached its maximum after elution with an additional 3,000 ml. of 0.1 N HCl, and then tapered off, no activity being present subsequent to elution with 4,000 ml. of 0.1 N HCl.
EFFECTS OF LIVER FRACTIONS ON MYOCARDIAL CONTRACTILITY

The active eluates were combined, taken to dryness in vacuo, brought to pH 10.9 with 100 ml. NH₄OH, filtered and put on a column of Dowex-1-Cl, 200-400 mesh, 2 per cent cross-linked. After elution with 1400 ml. of H₂O, the eluates began to show activity. One liter of 0.003 N HCl was then passed through the column. Activity continued to rise, reaching a maximum after 200 ml. had been collected and continuing through 350 ml. of eluate. After 1 L. of 0.003 N HCl had passed through, elution was carried out with 500 ml. of 0.01 N HCl, but additional activity was not obtained. The active eluates were combined and evaporated in vacuo. Yellow-white crystals were obtained. A portion was dissolved in water and tested on the isolated papillary muscle. It can be seen from Figure 10 that the effect induced by SMK/100 ml. of the extract was equivalent to that of C /µg./100 ml. of tyramine hydrochloride.

Another portion of the crystals was dissolved in water and chromatographed on Whatman no. 1 paper in two different solvent systems, (a) n-butanol:water:acetic acid, 50:25:25; (b) n-propanol:water:acetic acid, 70:20:10, in comparison with a sample of pure tyramine hydrochloride and in admixture with it. One chromatogram from each solvent system was sprayed with 0.5 per cent ninhydrin in 95 per cent ethanol, while the other was cut into one inch strips which were eluted with H₂O; the eluates were tested on the isolated papillary muscle. There was only one area of activity and this corresponded to the only ninhydrin positive spot. It had the same Rf value as tyramine. Both compounds decreased the threshold of excitability (e.g., 8.4 to 6.3 volts). The remainder of the impure crystalline extract was dissolved in ethanol, mixed with charcoal and filtered. The filtrate was placed in the refrigerator and the resultant white crystals were separated by filtration. They melted at 265 C. and when mixed with tyramine hydrochloride melted at 266 C. The tyramine hydrochloride itself melted at 266 C. Ultra-violet absorption spectra were run on pure tyramine and the isolated crystals; the spectra were superimposable with maxima at 2750 Å.

DISCUSSION

Liver Extracts. The substance with positive inotropic activity isolated from Expar E resembles tyramine in all measured respects. The two compounds have the same effects on the contractility of the isolated papillary muscle, have the same Rf values in two solvent systems, have the same ultra-violet absorption spectra and melt together and separately at the same temperature. It is concluded that the isolated compound is tyramine.

Free tyramine has not been shown to be present normally in mammalian tissues and the search for a tyrosine decarboxylase in such tissues also has been fruitless. In the absence of additional evidence, the presence of the free base in this liver fraction (Expar E) may be attributable to bacterial decarboxylation of tyrosine.

Pure Compounds. Of the many metabolites tested on the isolated papillary muscle, only methionine had activity. The positive inotropic effect was elicited at a concentration of 75 mg./100 ml., from 75 to 200 times its concentration in human plasma. The fact that both ethionine and the d-isomer of methionine were as effective as 1-methionine suggests that these were pharmacologic effects as distinct from phenomena related to the biochemical functions of 1-methionine. It may be fruitful to synthesize other sulfur-containing compounds and test them for action on the myocardium.

5-Hydroxytryptamine (5-HT) enhanced contractile power and excitability in a concentration about 25 times its level in normal blood and at about the same concentration reported in the blood of patients with malignant carcinoid. Since the lung traps 5-HT, it is likely that in malignant carcinoid with hepatic metastases, the right side of the heart receives a higher concentration of 5-HT than is present in the peripheral blood; and the amount striking the right side of the heart may even exceed that which is effective on the isolated myocardium. A peculiar aspect of the action of
5-HT is that at high doses it desensitized the muscle to previously effective doses. Others reported similar findings on the isolated ileum and atrium. No desensitization has been reported in the whole animal.

Menadione, the synthetic form of vitamin K, caused an increase in resting tension of the muscle with a concomitant decrease in contractile force and excitability leading to systolic arrest (fig. 8). Suspensions of vitamin $K_1$ and vitamin $K_1$-oxide were without any activity. Italian workers have reported similar observations on the hearts of frogs and rabbits in situ. They tested a series of naphthoquinones and found many to decrease contractile strength. Menadione has been shown to potentiate the effect of potassium on striated muscle, but this alone could not explain its cardio-toxicity, for high concentrations of potassium stop the mammalian heart in diastole.

The effect of Dicoumarol (fig. 9) was opposite to that of menadione. In vitro Dicoumarol dissociates oxidation from phosphorylation, but this could not be the basis for its cardio-toxicity because menadione in high concentrations has a similar effect on oxidative phosphorylation, although their effects on the heart are strikingly different.

**Summary**

Liver lecithin (in contrast with liver cephalin, brain lecithin, and brain cephalin) was found to contain a substance that increased contractile force of the isolated papillary muscle of the cat. This is probably the substance recently isolated and identified by others as palmitoyl lysolecithin.

Another compound with the property of enhancing the contractility of the papillary muscle was isolated from a liver fraction. This was crystallized and identified as tyramine.

Many compounds were tested for their effects on contractility of the myocardium. Of these, methionine at a concentration of 75 to 200 times its plasma level increased both contractility and excitability. The d- and l-isomers of methionine were equipotent. Ethionine did not antagonize the action of methionine; in fact, the activities of the compounds were identical.

Serotonin (5-hydroxytryptamine), at 25 times its normal concentration in blood, increased both contractility and excitability. The muscle showed desensitization: once exposed to high levels of the compound, the muscle no longer responded to previously effective low doses.

Heparin increased contractility in low doses and depressed contractility in high doses.

A lipid extract of liver containing steroids and glycerides depressed contractility and excitability. Of the steroids tested, only testosterone in high doses had such an effect, but unlike that of the liver extract its effect was not completely reversible.

None of the naturally occurring vitamins influenced contractility, but menadione stopped the muscle in systole. Dicoumarol, in contrast, stopped the muscle in diastole.

**Summario in Interlingua**

Esseva constatate que lecithina hepatic—per contrasto con cephalina hepatic, lecithina cerebral, e cephalina cerebral—contine un substantia que augmenta le fortia contractile del isolato musculo papilar del catto. Il es probabile che il se tracta hie del substantia recentemente isolate e identificate per alteres como lysolecithina palmitoylic.

Un alte composito capace a augmentar le contractilitate del muscolo papilar esseva isolate ab un fraction hepatic. Illo esseva crystallizate e identificate como tyramina.

Multe compositos esseva testate con respecto a lor effectos super le contractilitate del myocardo. Inter illos, methionina a una concentracion de 75 a 200 vices su concentracion in le plasma augmentava non solmente le contractilitate sed etiam le excitabilitate del myocardo. Le isomeros d-methionina e l-methionina esseva equipotent. Ethionina non esseva un antagonista del action de methionina. De facto, le activitate del duo compositos esseva identic.

Serotonina (5-hydroxytryptamina) a un concentration de 25 vices su concentration normal in le sanguine augmentava le contractilitate e
etiam le excitabilitate del myocardio. Le musculo manifestava dissensibilisation: Post su exposition a alte nivellos del composito, illo cessava responder a basse doses che habeva previamente essite efficace.

Heparina in basse doses augmentava le contractilitate e in alte doses deprimeva lo. Un extracto lipide de hepate, continente steroides e glyceridos, deprimeva le contractilitate e le excitabilitate. Inter le steroides testate, solmente testosterona in alte doses habeva iste mesme effecto, sed in le caso de testosterona—per contrasto con le extracto hepatic—le effecto non esseva completemente reversibile.

Nulle del vitaminas de occurrentia natural influenziava le contractilitate, sed menadiona arrestava le musculo in systole. Per contrasto con isto, dicoumarol arrestava le musculo in diastole.

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