body weight and less rapidly with larger doses. It was concluded from this study that previously reported estimates of labeling indices based on lower doses of administered H^3TDR are lower than the actual values. A dose of 1.0 μc H^3TDR/g body weight is recommended for the estimates of labeling indices using the "flash labeling" technique. However, doses of 1.0 μc and above are not to be used for cell migration or cell kinetic studies.

The authors wish to acknowledge the technical assistance of Miss M. Pavelec and Dr. B. Leventhal at NIH for the photomicrography.

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Received July 5, 1967. P.S.E.B.M., 1967, v126.

Influence of Positive Inotropic Agents on the Action of a Myocardial Depressant Factor in the Plasma of Cats in Postoligemic Shock.* (32438)

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Plasma of cats in irreversible postoligemic shock contains a myocardial depressant factor (MDF) which depresses the developed tension of isolated cat papillary muscles(1,2,3). The myocardial depression produced by MDF was completely reversed when the shock

* This investigation was supported, in part, by Nat. Heart Inst. Research Grants HE-01942 and HE-09924 and, in part, by a grant from the Virginia Heart Assn.

plasma was replaced with fresh Krebs-Henseleit solution. The purpose of the present study was to evaluate the ability of three types of positive inotropic drugs to reverse the negative inotropic effect of MDF. The drugs were a cardiac glycoside (ouabain), a beta adrenergic agent (norepinephrine), and a vasoactive peptide (angiotensin).

Methods. Shock procedure. Shock was induced by the method of Brand, *et al*(4) in healthy adult cats of either sex. The cats were anesthetized with pentobarbital (35 mg/kg, ip), heparinized (2250 units/kg), and bled from a femoral artery into a reservoir. The pressure in the reservoir was maintained between 40 and 45 mm Hg by bubbling 95% O_2 plus 5% CO_2 through the blood(5). When the cats had bled out maximally and then had spontaneously taken up from the reservoir 40% of the maximum bleeding volume, the remaining shed blood was reinfused. The rate of reinfusion was slowed when necessary to avoid a rise in central venous pressure (CVP) of more than 1 cm H_2O above the control value. Core body temperature was maintained at 35°C by warming the cat. Intermittent positive and negative pressure ventilation was maintained during the period of hemorrhagic hypotension. Ten to twenty minutes after reinfusion, the cat was exsanguinated from the cannulated femoral artery and the heart was removed. All hemodynamic variables were continuously recorded on a Grass model 5 polygraph with Statham pressure transducers used to measure MABP and CVP.

Papillary muscle preparation. Adult cats were anesthetized with ether followed by pentobarbital sodium (25 g/kg, iv). The papillary muscles were excised from the right ventricle and placed in 20-ml chambers in a modified Krebs-Henseleit solution(5), gassed with 95% O_2 + 5% CO_2 and maintained at 37 ± 0.1°C. The Krebs-Henseleit solution contained 2.54 mM $CaCl_2$ rather than 0.63 mM $CaCl_2$, as was used in our previous study with ouabain(3). Threshold stimulating voltages and active length-tension relationships were determined for each muscle. Individual muscles were stimulated at a frequency of 60/min, with a stimulus of 16.7 msec duration and a strength of 2 v above the threshold voltage. Resting tension was set at a point about 80% of that yielding maximum developed tension. Inotropic responses were calculated as changes in developed tension in g/mm^2 and are expressed as per cent change above pre-drug developed tension. The drug concentrations used throughout these studies were as follows: ouabain (1.74×10^{-7} M), norepinephrine (7.5×10^{-9} M), and angiotensin (1.25×10^{-8} M).† All drugs were made up fresh daily, diluted in Krebs-Henseleit solution, and added to the bath in a volume of 0.1 to 0.5 ml.

Results. Effect of control and shock plasma on developed tension. The inotropic effects of the 3 media used are summarized in Table I. There was no significant difference between the developed tension of muscles removed from normal control anesthetized cats (control muscles) and muscles removed from cats early in the normovolemic normotensive stage of irreversible postoligemic shock (shock muscles) when the muscles were bathed in Krebs-Henseleit solution. However, normal control cat plasma (control plasma) decreased developed tension both in control muscles and in muscles from cats in early shock. This depression was statistically significant when analyzed using each muscle as its own control. There was no significant difference in the magnitude of the decrease in developed tension in control *vs* shock muscles exposed to control plasma. Furthermore, plasma removed from cats early in the normovolemic normotensive stage of irreversible postoligemic shock (shock plasma) depressed developed tension to a significantly greater degree than did control plasma in both groups of muscles ($p<0.001$). Shock plasma depressed shock muscles to a significantly greater degree than it did control muscles ($p<0.005$).

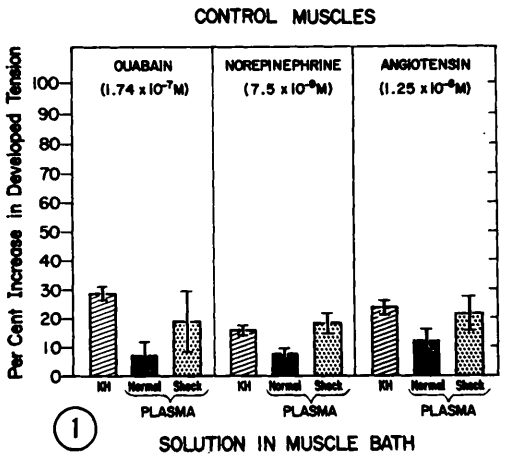
Effect of control and shock plasma upon the responsiveness to inotropic agents. The inotropic responses to the 3 drugs tested are summarized in Fig. 1 (control muscles) and in Fig. 2 (shock muscles). These figures represent the basic data showing the inotropic

† Ouabain (Lilly), norepinephrine (Levophed, Sterling-Winthrop, and angiotensin (Hypertensin, Ciba.)

TABLE I. Developed Tension in Papillary Muscles Bathed in 3 Media.

Solution	Measurement	Control muscles	(N)	P	Shock muscles	(N)	P
KH	Developed tension (g/mm²)	1.42 ± .06	(32)	—	1.48 ± .10	(31)	—
Normal plasma	Change from KH (%)	−12.5 ± 6.1	(13)	<.05	−20.0 ± 4.7	(22)	<.001
Shock "	" " " "	−32.8 ± 5.1	(13)	<.001	−49.9 ± 3.0*	(22)	<.001

(N) = number of muscles tested.
* Shock muscles were depressed more than were normal muscles ($p<.005$).



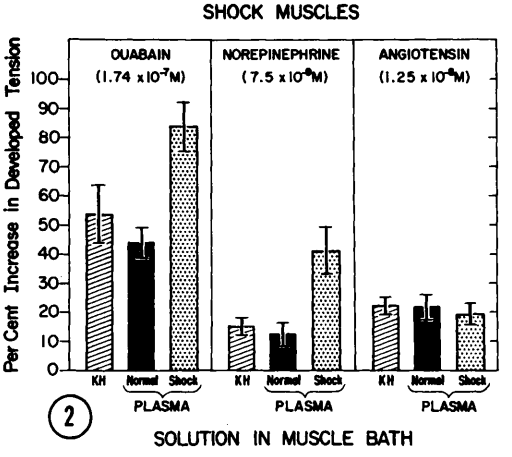


FIG. 1. Inotropic responsiveness of control muscles to ouabain, norepinephrine and angiotensin. Each bar represents the mean per cent increase in developed tension ± SEM for 8 muscles. The first bar in each group is the response in Krebs-Henseleit solution, the second bar, normal plasma, the third bar, shock plasma.

FIG. 2. Inotropic responsiveness of shock muscles to ouabain, norepinephrine and angiotensin. Each bar represents the mean per cent increase in developed tension ± SEM for at least 9 muscles.

responsiveness of both control and shock papillary muscles in the three media used.

TABLE II. Comparison of Inotropic Responsiveness of Control and Shock Papillary Muscles to Inotropic Agents.

	Krebs-Henseleit solution	Control plasma	Shock plasma
Ouabain	1.9*	6.6‡	4.6‡
Norepinephrine	1.0	1.6	2.6†§
Angiotensin	.9	1.8	.9

All values expressed as ratio of responsiveness of shock muscles compared with control muscles expressed as a decimal equivalent.
* p .025
† p .01
‡ p .001
§ Response in shock plasma significantly greater than in control plasma when calculated as per cent change from pre-drug control ($p<.005$).

Table II shows the response of the shock muscles expressed as the ratio of their response to that of the normal muscles under the same experimental conditions. It is evident from Table 2 that shock muscles responded to a greater degree than did control muscles to ouabain in Krebs-Henseleit solution ($p<0.025$). No significant differences however, existed between control and shock muscles in response to angiotensin or to norepinephrine when added to the KH solution.

Control plasma decreased the inotropic responsiveness of the control papillary muscles to all 3 drugs in comparison to the responsiveness in Krebs-Henseleit solution. The response to ouabain was decreased more than that to norepinephrine ($p<0.005$) but not to angiotensin. This decreased responsiveness to inotropic agents in control plasma did not occur in shock muscles. Shock muscles showed a greater inotropic responsiveness to ouabain than did control muscles bathed in control plasma ($p<.001$).

Shock plasma, containing MDF, generally increased the responsiveness of both types of muscles to ouabain and to norepinephrine,

compared to the responsiveness in control plasma. This increased responsiveness was statistically significant in the case of the response of the control muscles to norepinephrine ($p<0.05$), and in the response of the shock muscles to ouabain ($p<0.001$) and to norepinephrine ($p<0.005$). Shock muscles showed a greater response to ouabain ($p<0.005$) and to norepinephrine ($p<.01$), than did control muscles when bathed in shock plasma.

Ouabain or norepinephrine, when added to shock plasma, increased the absolute developed tension of shock muscles to within the range of the same shock muscles stimulated by these drugs while immersed in normal plasma. Thus, the myocardial depressant effect of shock plasma was completely reversed in shock muscles by both ouabain and norepinephrine. None of the 3 drugs tested completely reversed the myocardial depressant effect of MDF in control muscles, in contrast to shock muscles.

Angiotensin did not produce a significantly greater response in shock plasma than in control plasma, nor was there a significant difference between the response of shock muscles and of control muscles to angiotensin in any of the three media.

Discussion. These data show that ouabain and norepinephrine can completely reverse the negative inotropic effect of MDF in shock muscles. These findings suggest that cardiac glycosides or beta adrenergic agents may be useful, in addition to removal of the MDF(4), in the therapy of "irreversible" shock. This suggestion is based upon the hypothesis that depression of myocardial contractility by MDF is a significant factor in the cardiovascular failure of irreversible shock. Indeed improved survival has been reported in shocked animals treated with cardiac glycosides(6,7,8) and with beta adrenergic agonists (*i.e.*, isoproterenol and nylidrin)(9).

The enhanced responsiveness of shock muscles to ouabain and norepinephrine, in the presence of MDF, suggests that MDF does not depress contractility in shock muscles by blocking those receptors which are activated by cardiac glycosides or by beta adrenergic drugs.

The finding of several significant differences between shock and control muscles suggests that the heart, even in the early normotensive stage of postoligemic shock, is functionally altered. The fact that shock muscles are more severely depressed than control muscles by shock plasma suggests that this altered function may be a significant factor in the pathogenesis of shock.

The mechanism by which normal plasma decreased inotropic responsiveness of normal muscles to all 3 drugs tested is not known. However, plasma binding of ouabain is probably not a factor because it is virtually nonexistent(10,11)

The depressant effect of control plasma on developed tension of papillary muscles has been described by us previously(1,3), and can be attributed to the presence of pentobarbital in the plasma, and to a slightly lower concentration of free calcium in the plasma than is present in Krebs-Henseleit solution.

Summary. Ouabain, norepinephrine, and angiotensin were added to papillary muscles removed from normal cats and from cats in shock. Solutions used in the muscle bath were Krebs-Henseleit solution (KH), normal plasma and shock plasma which contained a myocardial depressant factor (MDF). The MDF depressed developed tension in shock muscles by 50% of the value in KH while it only depressed control muscles by 33%. Ouabain and norepinephrine, but not angiotensin, completely reversed the effect of MDF in shock muscles, restoring contractile force to that present in control plasma. Shock muscles differed from control muscles in that they were more depressed by MDF, and they were more responsive to ouabain. Thus, shock muscles, although functionally altered and depressed by MDF, respond well to ouabain and to norepinephrine. Therefore, cardiac glycosides and β adrenergic drugs may be useful in the therapy of shock.

The technical assistance of Mr. Thomas F. Inge, Jr., and Mr. Christie W. Winkler is gratefully acknowledged.

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Received June 15, 1967. P.S.E.B.M., 1967, v126.

Effect of Chlorpromazine on Rat Fat Metabolism. (32439)

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Since the advent of phenothiazine therapy chronic administration of chlorpromazine (CPZ) in man has been noted to be associated with an increase in body weight(1,2). Two different mechanisms have been suggested as causes of this phenomenon. Hülsmann *et al* (3) demonstrated that CPZ *in vitro* inhibited the ability of lipases from lung and adipose homogenates of the rat to produce glycerol from fat, and suggested that this inhibition might be responsible for the marked increase in weight seen in patients receiving this drug. Recently studies from two laboratories demonstrated that high doses of CPZ (5×10^{-3} to 1×10^{-3} M) *in vitro* reduce the norepinephrine (NE) induced free fatty acid (FFA) release from rat epididymal fat pads (4-6). This inhibition of FFA release might also be interpreted as contributing to adiposity, but such a mechanism has not been shown to be associated with the *in vivo* administration of CPZ.

In addition to weight gain CPZ administration is known to produce hypothermia in various experimental animals(7-10). The degree of hypothermia increases as the ambient temperature falls(9,11,12), but the temperature of the animals will remain normal at approximately 31°C environmental temperature(13). Hyperglycemia(11), decreased oxygen consumption(8), decreased glucose consumption(11), and inhibition of protein synthesis *in vivo*(12) seen after acute CPZ administration are secondary to this hypothermia and return to normal with proper maintenance of the animals' temperatures.

The present paper presents studies on the effect of *in vivo* administration of CPZ on glucose uptake and glycerol release *in vitro* by rat epididymal adipose tissue from donors with and without hypothermia. Data are provided which demonstrate that the hypothermia induced by the *in vivo* administration of CPZ in rats is associated with increases in fat pad weight and glucose uptake, and decreases in glycerol release.

Methods. Male white Charles River rats weighing between 200-300 g were fed rat chow pellets (Purina) and tap water *ad libitum*. Animals were housed either in pairs in wire cages (8″ × 13½″ × 6″) or individually in plastic cages (5″ × 6″ × 10½″). Each animal was weighed and handled at least 3 days prior to each experiment. All animals were housed in our facilities for at least 6 days prior to the studies on a light-darkness alternated 12 hr schedule. Each study was initiated at 9:30 to 11 a.m. when, after weighing, the animals were injected i.p. with either 20 mg of CPZ per kg body wt (Thorazine®, Smith, Kline and French*) in commercial vehicle or the vehicle without CPZ, and food was removed for those animals scheduled for fasting. CPZ and placebo injections were repeated daily at 9:30 to 11

* The authors are indebted to Smith, Kline and French Co. for the gift of a generous amount of CPZ and placebo vehicle.