Cardiac Glycogen Fractions in the Ventricles of Fasted and Non-Fasted, Exercised Rats

Previous workers, using in vitro techniques and reporting total glycogen concentrations, have failed to demonstrate differences in glycogenolysis between the left and right ventricles[1,2]. In an earlier work[3] it was pointed out that non-fasted rats forced to swim for varying periods of time depleted their left ventricular glycogen stores. The glycogen concentrations in the swimming experiments were reported as TCA glycogen (obtained from the supernatant of a trichloroacetic acid homogenate) and residual glycogen (found in the protein precipitate of the centrifuged homogenate). The swimming experiments did not include glycogen analysis of the right ventricle.

The present experiment was designed to explore differences in right and left ventricular glycogenolysis in each glycogen fraction produced by swimming. In addition the experiment was constructed to determine if the method of processing tissue influenced the glycogen concentrations of one ventricle more than the other. BLOOM[4], using the whole heart, detected no glycogen decrease in the excised organ left in room air or nitrogen for 4 min. In the present work glycogen concentrations in left and right ventricles that had been rapidly frozen were contrasted with ventricles that were weighed and transferred to the homogenizing tube in the non-frozen state.

Methods and materials. Non-fasted and fasted male albino rats (Sprague-Dawley strain), weighing between 130 and 290 g, were used in the experimental procedures. The non-fasted animals (87 rats) were given free access to Purina Laboratory Chow and water, and the fasted animals, previously fed Purina Laboratory Chow (83 rats) were not allowed food 24 h prior to sacrifice. Experimental (exercised) and control (rested) animals were drawn from each new population of rats to insure adequate sampling of the population, and they were sacrificed either late in the morning or early in the afternoon. All animals were kept in the animal quarters at least 1 week prior to sacrifice.

Glycogen was obtained from the cardiac tissue in 2 fractions according to the method of BLOOM et al.[5]. Both the trichloroacetic acid (TCA) and residual fractions were analyzed by the anthrone method of SEIFTER et al.[6]. The glycogen values are reported in mg of glycogen/100 g of wet tissue.

In order to determine not only the effect of exercise but also the effect of method of handling the tissue on the glycogen concentrations 2 large groups of rats were studied. Within each group the glycogen fractions were obtained in right and left ventricles of fasted and non-fasted animals after swimming exercise.

In the first group non-fasted and fasted animals were forced to swim for a 15 min period in water at 25 °C. The animals were beheaded immediately after swimming, and each heart was rapidly excised, blotted, and separated into left ventricle with interventricular septum attached (henceforth referred to as the left ventricle) and free right ventricular wall. The right ventricle was immediately placed on aluminium foil and frozen between cakes of dry ice. The left ventricle was weighed and transferred to a Ten-Broeck tissue grinder for homogenization in cold 10% trichloroacetic acid. The frozen right ventricle was then weighed and also homogenized. Removal of the heart, trimming and separating the ventricles required approximately $1^1/_2$–2 min. The difference in time between freezing the right ventricle and weighing and placing of the left ventricle in the tissue grinder for homogenizing was from 30–60 sec. The total time between beheading the rat and homogenizing the non-frozen tissue was not greater than $2^1/_2$ min. This delay of $2^1/_2$ min undoubtedly allowed additional glycogenolysis to occur, but comparisons could be made between the various categories of animals by keeping the isolation time constant for the right and left ventricles.

A second group of animals was sacrificed, and all the experimental variables were identical to the first group with the exception that the freezing procedure was reversed. The left ventricle was frozen first and the right ventricle processed without freezing. Hereafter, the cardiac tissue will be referred to either as frozen or non-frozen, indicating which method was used in handling the ventricles.

Statistical analyses were carried out using the least squares analysis of variance and the student *t* test.

In Figure 1 glycogen values were pooled according to several different criteria and the individual plots tested for significance. A glycogen value may be TCA or residual, belong to a left or right ventricle that was frozen or non-frozen after being removed from a fasted or non-fasted animal. In order to present the data in a more concise and meaningful way the above information was coded on IBM cards and pooled so that any one analysis of variance plot would contain only 2 identifying bits of information. For example, the adjusted means of all glycogen values (regardless of whether TCA or residual) of left and right ventricles from animals forced to swim were compared with all glycogen values of left and right ventricles from animals in the rested state in Figure 1C.

Results. All the data of the experiment are presented in the Table and organized according to method of handling

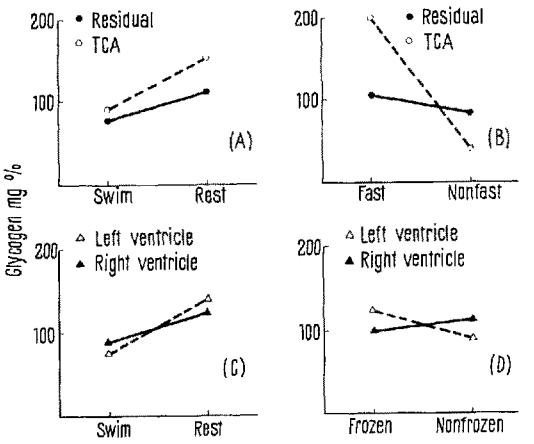

Fig. 1. Two-way interactions based on least squares analysis of variance. (A) Residual and TCA glycogen fractions in the swimming and rested state, F = 17.31. (B) Residual and TCA glycogen in fasted and nonfasted rats, F = 380.78. (C) Left and right ventricles in the swimming and rested state, F = 12.90. (D) Left and right ventricles in frozen and non-frozen conditions, F = 41.49. All above F values have a probability value of less than 0.001.

1 L. A. JEDEIKIN, Revue can. Biol. *22*, 165 (1963).
2 L. SZEKERES and G. LICHNER, Acta physiol. hung. *21*, 243 (1962).
3 D. H. BLOUNT and D. K. MEYER, Am. J. Physiol. *197*, 1013 (1959).
4 W. L. BLOOM, Am. J. Physiol. 186, 518 (1956).
5 W. L. BLOOM, G. T. LEWIS, M. Z. SCHUMPERT and T. SHEN, J. biol. Chem. *188*, 631 (1951).
6 S. SEIFTER, S. DAYTON, B. NOVIC and E. MUNTWYLER, Archs Biochem. *25*, 191 (1950).

Ventricular glycogen fractions according to method of handling tissue

	Left ventricle			Right ventricle		
	Observations	Non-frozen	Frozen	Observations	Non-frozen	Frozen
Fasted						
Residual						
Swim	25, 17	89 ± 6[a]	109 ± 5[a]	14, 24	100 ± 5	92 ± 5
Rest	21, 17	111 ± 8[a]	132 ± 4[a]	16, 21	118 ± 5	103 ± 6
TCA						
Swim	24, 18	104 ± 11[a]	165 ± 16[a]	19, 24	182 ± 13	164 ± 11
Rest	19, 18	232 ± 19	282 ± 21	18, 21	252 ± 20	220 ± 18
Non-fasted						
Residual						
Swim	31, 12	48 ± 5[a]	73 ± 6[a]	12, 30	63 ± 6	61 ± 6
Rest	25, 11	83 ± 7[a]	154 ± 6[a]	12, 32	121 ± 5[a]	77 ± 4[a]
TCA						
Swim	31, 12	26 ± 4	17 ± 2	12, 29	22 ± 2	36 ± 6
Rest	31, 12	49 ± 4[a]	70 ± 8[a]	12, 32	54 ± 5	56 ± 6

Glycogen values (mean ± s. e. of mean) in mg/100 g of wet tissue. [a] Probability value of less than 0.036.

the tissue. In the left ventricle 6 of the 8 conditions display significantly lower glycogen values for the non-frozen compared with the frozen tissue. In the right ventricle only one significant difference is noted, and that difference reveals a glycogen value higher for the non-frozen than for the frozen tissue.

In Figure 1 the significant two-way interactions are plotted. All of the pairs of adjusted means presented in each plot are significantly different from each other with a P value of less than 0.001. In Figure 1A the plot indicates that the TCA fraction of glycogen was depleted more in swimming than was the residual fraction. (These data are further analyzed in Figure 2). Figure 1B shows that the TCA fraction was elevated considerably more than the residual fraction under the fasted condition. The influence of swimming exercise on the glycogenolytic activity of the 2 ventricles is reflected in the plot of Figure 1C. The left ventricular glycogen was depleted more than the glycogen of the right ventricle as a result of the swimming experience. In Figure 1D the analysis of variance plot reveals the difference in glycogen values when the 2 tissues were either frozen and then homogenized or allowed the 30–60 sec delay between separation and homogenization. Glycogenolysis in the left ventricle was more influenced by the method of handling the tissue than in the right ventricle.

The data pooled in the three-way interaction presented in Figure 2 show the comparison between glycogen fractions of animals exercised and rested in the fasted and non-fasted condition. This plot contrasts the significance of the difference between the 2 pairs of data, and it reveals that the TCA fraction was utilized much more in the fasted condition than was the residual fraction ($P < 0.001$). In the non-fasted condition both fractions were utilized more equally when the animals were forced to swim.

Discussion. In the pooled data the TCA fraction was shown to be more depleted than the residual fraction due to swimming exercise, and in fasted animals the TCA fraction was elevated much more than the residual fraction. Both of these facts have been pointed out in the previous literature[7,8]. It has not been demonstrated previously that fasted animals use more TCA than residual glycogen when forced to swim, whereas non-fasted animals use both fractions more equally. This result is probably due to the initial limited quantity of TCA glycogen in the hearts of the rested, non-fasted animals and implies a preference for the TCA glycogen if it is present in sufficient quantity.

It is probable that the difference in glycogenolysis in the ventricles is related to a greater imbalance between oxygen supply and metabolic needs in the left ventricle than in the right ventricle. Even though SZEKERES and LICHNER[2] were unable to show different glycogenolytic rates between left and right ventricular tissue samples incubated for 1 h they did demonstrate that left ventricular QO_2 was significantly greater than that found in right ventricular tissue. This greater oxidative demand by the left ventricle would play a significant role in producing greater glycogenolysis in the left ventricle during exercise.

The difference between the glycogen concentrations of frozen tissue and non-frozen tissue is consistent with the

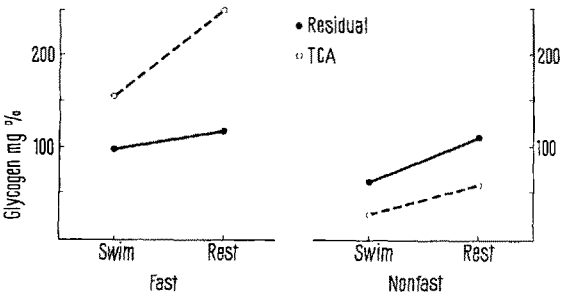

Fig. 2. Three-way interaction based on least squares analysis of variance. Residual and TCA glycogen fractions in fasted and non-fasted, exercised and rested animals, $F = 40.65$, $P < 0.001$.

[7] G. A. ADROUNY and J. A. RUSSELL, Endocrinology *59*, 241 (1956).

QO_2 data presented by SZEKERES and LICHNER. If the left ventricle has a greater rate of aerobic metabolism than the right ventricle one would expect the left ventricle to be more sensitive to the isolation procedures. The inability of SZEKERES and LICHNER to show differences in rates of glycogenolysis in their incubation studies is probably due to the prolonged time interval used to study this process[8,9].

Zusammenfassung. Vergleich von Glykogenfraktionen aus Ventrikeln männlicher Albinoratten, die 24 h gefüttert wurden, mit Nichtgefütterten. Letztere verbrauchten meistens TCA (ungebundenes Glykogen) nach Bewegung, während gefütterte Ratten sowohl Rest-Glykogen (gebundenes) wie auch TCA Glykogen verbrauchten. Die Glykogenabnahme war im linken Ventrikel nach Bewegung grösser als im rechten.

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Über die amindepletierende Wirkung verschiedener Digitaliskörper, ein Beitrag zur Aufklärung des Wirkungsmechanismus der Herzglykoside

Der Hauptangriffspunkt der Digitaliskörper wird von vielen Autoren im Kalziumstoffwechsel der Herzmuskelzelle vermutet (Übersicht bei KLAUS[1]). Durch eine Beeinträchtigung der Rückbindung des während der Erregung intrazellulär freigesetzten Kalziums oder über einen Angriff an der Transport-ATPase[2] soll die Zunahme der biologisch aktiven Kalziumfraktion zu einer stärkeren Hemmung des Erschlaffungsfaktors und damit zu dem positiv inotropen Effekt führen.

Eine Anzahl von Befunden der letzten Jahre[3–13] unterstützt die zuerst von TANZ[4] geäusserte Vermutung, dass ein Teil der Herzglykosidwirkung über eine Katecholaminfreisetzung aus dem Herzmuskel zu erklären sei, da eine durch Reserpin oder Guanethidin bedingte Amindepletion bzw. eine Vorbehandlung mit β-Rezeptorenblockern den positiv inotropen Digitaliseffekt abschwächen. In eigenen Untersuchungen[12] konnten wir nach i.p. Gabe hoher Dosen von g-Strophanthin, Digitoxin und Digitoxigenin innerhalb der Fehlerbreite der Methodik im Herzmuskel keine statistisch zu sichernde Noradrenalinverminderung feststellen. CESSION-FOSSION[14] dagegen, dessen Arbeit uns erst nach Abschluss unserer Versuche bekannt wurde, und LOUBATIÈRES et al.[15] fanden nach i.v. Gabe niedriger g-Strophanthindosen an Ratten oder nach i.p. Gabe an Meerschweinchen eine statistisch zu sichernde Verminderung des Noradrenalingehaltes des Herzens. Zur Klärung der Diskrepanzen zwischen den verschiedenen Befunden führten wir mit mehreren Glykosiden Nachuntersuchungen durch.

Die Digitaliskörper wurden Ratten i.v. in Dosen verabreicht, die keine deutlichen extrakardialen Erscheinungen hervorriefen. Die Aminveränderungen im Herzen bezogen wir auf die täglich mit den entsprechenden Dosen von Äthanol als Lösungsmittel injizierten Kontrolltiere. Um die Reproduzierbarkeit der Ergebnisse zu kontrollieren, wurden einige Glykoside an mehreren Tagen in der gleichen Dosis appliziert. Die Aminbestimmungen erfolgten spektrofluorometrisch modifiziert nach ANTON und SAYRE[16] und COHEN und GOLDENBERG[17], die statistische Auswertung wurde varianzanalytisch oder nach WINNE[18] durchgeführt. Die Ergebnisse sind in der Tabelle gegenübergestellt. Aus der Tabelle ergibt sich, dass an der Ratte in dem geprüften Dosenbereich alle untersuchten herzwirksamen Steroide eine statistisch zu sichernde Aminverarmung im Herzmuskel hervorrufen. Die Stärke der Aminverarmung variiert mit der Zeitdauer der Einwirkung und der Dosis, scheint jedoch auch nach hohen Dosen 50% nicht zu übersteigen. Als Ergänzung zu unseren früheren Versuchen wurde Ratten 50 mg/kg g-Strophanthin i.p. injiziert und die Einwirkungszeit auf 60 Minuten verlängert. Trotz der hohen Dosis kam es nur zu einer angedeuteten Aminverarmung ($P < 0{,}05$).

Unsere Befunde bestätigen die Ergebnisse von CESSION-FOSSION und LOUBATIÈRES über eine Noradrenalinverarmung des Herzens nach Gabe von Digitaliskörpern. Ob der Effekt auf eine Steigerung der Aminfreisetzung oder Hemmung der Aminsynthese zurückzuführen ist, können erst weitere Untersuchungen

[1] W. KLAUS, Zschr. naturw.-med. Grundlagenforsch. *2*, 43 (1964).
[2] K. REPKE, 2. Ungarische Konferenz f. pharmakol. Forsch. 1962, Budapest (1964), S. 300.
[3] K. PÁVEK und F. V. SELECKÝ, Bratisl. lek. Listy *40*, 481, 551 (1960).
[4] R. D. TANZ, Pharmacologist *2*, 95 (1960).
[5] V. CAIROLI, J. RELLY und J. ROBERTS, Fedn Prod. Fedn Am. Socs exp. Biol. *20*, 122 (1961).
[6] A. WOLLENBERGER und W. HALLE, Mber. dt. Akad. Wiss. Berl. *5*, 38 (1963).
[7] W. FÖRSTER und U. STOLZENBURG, Acta biol. med. germ. *11*, 86 (1963).
[8] W. SZIEGOLEIT und W. FÖRSTER, Experientia *20*, 229 (1964).
[9] R. D. TANZ, J. Pharmac. exp. Ther *144*, 205 (1964).
[10] R. D. TANZ und S. M. MARCUS, J. Pharmac. exp. Ther. *151*, 38 (1966).
[11] A. P. LOUBATIÈRES, P. BOUYARD, M. KLEIN, J. CHAPAL, A. M. RONDOT und L. CAUSSEDIER, C. r. Séanc. Soc. Biol. *159*, 953 (1965), zit. nach Excerpta med. IIC/*19*, 314 (1966).
[12] W. FÖRSTER und V. RÖSLER, Acta biol. med. germ. *16*, 309 (1966).
[13] W. FÖRSTER und H. KALSOW, Acta biol. med. germ. *15*, 71 (1965).
[14] A. CESSION-FOSSION, C. r. Séanc. Soc. biol. *156*, 1192 (1962).
[15] A. LOUBATIÈRES, P. BOUYARD, J. CHAPAL, M. KLEIN und A. M. RONDOT, C. r. Séanc. Soc. Biol. *159*, 948 (1965), zit. nach Excerpta med. IIC/*19*, 314 (1966).
[16] A. H. ANTON und D. F. SAYRE, J. Pharmac. exp. Ther. *145*, 326 (1964).
[17] G. COHEN und M. GOLDENBERG, J. Neurochem. *2*, 71 (1957).
[18] D. WINNE, Arzneimittel-Forsch. *14*, 62 (1964).