of VESSMAN et al.[6]. The TMS and HFB derivatives were run on 3% SE-30, 6 ft column under temperature programming along with even-numbered hydrocarbon mixture (C_{12}–C_{24}). The methylene unit values were calculated and compared with standard reference compounds.

Results and discussion. The identification of the amines was considered positive only when GLC and at least one of the 2 other methods used showed positive tests. N-dimethyltryptamine and 5-methoxy-N-dimethyltryptamine were identified in all 5 of the acute schizophrenic patients both in the free and conjugated amine fractions; bufotenin was identified in the free amine fraction in only 2 acute patients. In the chronic schizophrenics, the 2 normals and 1 depressive, the N-dimethyltryptamines could not be identified by the above criteria. The TMS and HFB derivatives of the free amine fractions, which were identified by GLC, showed peaks having the same methylene unit (MU) values as standard N-dimethyltryptamine and 5-methoxy-N-dimethyltryptamine and bufotenin. In the course of our investigations on the urinary excretion of dimethylated indoleamines in schizophrenia, we also examined the urine samples of 2 of the 5 acute schizophrenics described in this study. N-dimethyltryptamine, 5-methoxy-N-dimethyltryptamine and bufotenin were identified in the free amine fractions in the urine samples of both patients, by two-dimensional thin-layer chromatography, as well as by gas-liquid chromatography. The latter results will be published in a separate communication.

Resumen. En nuestros estudios en sangre de esquizofrenicos agudos, hemos encontrado, usando cromatografia en capa delgada y cromatografia gaseosa liquida, dimetiltriptamina, y 5-metoxi-N,N-dimetiltriptamina. En solo uno de ellos hallamos bufotenina. Estas substancias no fueron detectadas en nueve esquizofrenicos cronicos, dos normales y un enfermo depresivo.

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[6] J. VESSMAN, A. M. MOSS, M. G. HORNING and E. C. HORNING, Analyt. Letters *2*, 81 (1969).

LDH Isoenzyme Spectrum in the Myocardium of Rats after Repeated Doses of Isoproterenol

When isoproterenol is administered in large doses it causes cardiac necrosis[1,2]. If, however, it is given in small doses for several days, there is an increase in the resistance of the myocardium to necrogenic doses of this catecholamine[3], and at the same time an increase in the tolerance of the right ventricle to acute anoxia[4].

Isoproterenol cardiopathy is accompanied by a marked shift in the LDH isoenzyme spectrum of the myocardium in favour of an anaerobic type of metabolism[5,6]. We therefore raised the question of whether one of the factors responsible for increased tolerance of the heart to acute anoxia and isoproterenol necrosis in rats adapted to small doses of isoproterenol is a changed capacity for anaerobic glycolysis.

Methods. The experiments were made on Wistar rats, average weight 250 g, fed on a normal laboratory diet with water ad libitum. Isoproterenol (Spofa) in doses of 1 mg/kg body weight (6 × mg/kg) was injected s.c. at 24h intervals for 6 days. 2 days after the last dose of isoproterenol, the rats were decapitated and enzymes determined in the entire myocardium.

Cardiac tissue was homogenized at 0 °C in veronal buffer at pH 8.4 and centrifuged at 15,000 rpm for 30 min, LDH isoenzymes were determined in the supernatant by electrophoresis in agar[7]. Isoenzymatic activity was detected by nitroblue tetrazolium, evaluated on an ERI 10-Zeiss Jena densitometer, and the curves constructed by planimetry. The proportion of the separate isoenzymes was expressed as a percentage of the total area of the curve. The percentage of M-units was calculated on the basis of the representation in the individual isoenzymes[8]. Total lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activity were measured spectrophotometrically by observing decrease of extinction at 340 nm and the specific activity expressed in μM NADH/h/g wet weight.

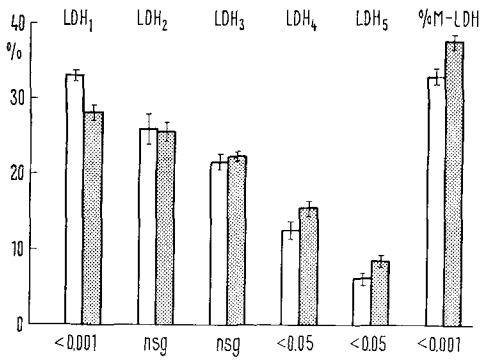
Results. The myocardium of rats which had received injections of isoproterenol for 6 days showed a marked shift of the lactate dehydrogenase isoenzyme spectrum towards slower isoenzymes of the M-type (Figure). LDH_1 values were decreased ($P < 0.001$), whereas LDH_4 and LDH_5 were significantly increased. These changes clearly reflect an increased proportion of M-subunits of LDH, which is evidence that there was a decrease in the type of metabolism obtaining energy via oxidative cycles in favour of the type of metabolism with energy formation by anaerobic glycolysis. Total LDH and MDH activity remained unchanged.

Discussion. These results show that in rats adapted to small doses of isoproterenol there is an increase in the ratio of anaerobic to aerobic type of LDH isoenzymes. The increased anaerobiosis is probably one of the factors responsible for the greater resistance of these rats to acute

[1] C. J. CHAPPEL, G. RONA, T. BALASZ and R. GAUDRY, Archs. int. Pharmacodyn. Thér. *122*, 123 (1959).
[2] G. RONA, C. J. CHAPPEL, T. BALASZ and R. GAUDRY, Arch. Path. *67*, 443 (1959).
[3] Z. TUREK, M. KALUŠ and O. POUPA, Physiologia bohemoslov. *15*, 353 (1966).
[4] O. POUPA, Z. TUREK, V. PELOUCH, J. PROCHÁZKA and K. KROFTA, Physiologia bohemoslov. *14*, 536 (1965).
[5] D. G. WENZEL and J. P. LYON, Toxic. appl. Pharmac. *11*, 215 (1967).
[6] M. JELÍNKOVÁ and E. FALTOVÁ, Physiologia bohemoslov., in press (1970).
[7] R. J. WIEME, *Studies on Agar Electrophoresis. Technique-applications* (Arocia Nitgaven N.V., Brussel 1959).
[8] E. B. THORLING and K. JENSEN, Acta path. microbiol. scand. *66*, 426 (1966).

anoxia. Data in the literature[9] show that after the administration of isoproterenol there is an increase in the myocardium in the level of the energy substrate, glycogen, and also of Q_{O_2}[10] but not in correlation with resistance of the heart to acute anoxia.

Increased resistance both of the myocardium to anoxia in vitro, and to necrogenic doses of isoproterenol was also found in height adapted rats[11]. Anaemic rats also have a greater tolerance for anoxia[12]. One of the common factors in all 3 models (adaptation to isoproterenol, adaptation to height[13], anaemic rats[14] is a shift of LDH isoenzymes in favour of the anaerobic type of metabolism which is correlated with increased tolerance of the myocardium for acute anoxia in vitro. An increase in M-type LDH may be one of the mechanism by which the heart becomes adapted to an anaerobic environment. An increase in M-type subunits can maintain LDH activity, even in the presence of a greater amount of substrate and can thus maintain the heart in a state of greater efficiency during anoxic periods in experiments in vitro and on the isolated right ventricle[11].



Distribution of isoenzymes LDH and percent of M-type subunits LDH in the heart. □, controls; ////, isoproterenol.

Zusammenfassung. Die an kleine Dosen Isoproterenol gewöhnten Herzen von Ratten wiesen eine bedeutsame Vermehrung der LDHM-Untereinheiten auf. Die Zunahme der Fähigkeit zu anaërober Glykolyse ist wahrscheinlich einer der Faktoren, der für die grössere Widerstandsfähigkeit der isolierten rechten Herzkammer dieser Ratten gegen akute Anoxie verantwortlich ist.

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Czechoslovak Academy of Science,
Praha (Czechoslovakia), 2 December 1969.

[9] O. Poupa, J. Procházka and V. Pelouch, Physiologia bohemoslov. *17*, 36 (1968).
[10] O. Poupa, Z. Turek, V. Pelouch, J. Procházka and K. Krofta, Physiologia bohemoslov. *14*, 536 (1965).
[11] O. Poupa, K. Krofta, J. Procházka and Z. Turek, Fedn. Proc. *25*, 1243 (1966).
[12] O. Poupa, K. Rakušan, J. Procházka and K. Krofta, Physiologia bohemoslov. *14*, 452 (1965).
[13] M. Mager, W. F. Blatt, P. S. Natale and C. M. Blatteis, Am. J. Physiol. *215*, 8 (1968).
[14] M. Jelínková, J. Souhrada and O. Poupa, Physiologia bohemoslov. *17*, 467 (1968).

The Relationship Between Activation Heat and Calcium Transients in Frog Sartorius Muscle

The heat which antecedes tension development has been originally defined by Hill[1] as activation heat; this heat is still produced after work and tension development is eliminated. During the initial events preceding contraction, the activator calcium is released from the sarcoplasmic reticulum[2].

The relation between the kinetics of Ca^{2+} changes and the time course of heat liberation at 0 °C has been studied in freshly excised frog sartorius muscle, using the characteristic absorption of the Ca-murexide complex at 470 nm and 540 nm as a measure of the free Ca^{2+} concentration. The procedure was that of Jöbsis and O'Connor[3]. To eliminate any mechanical response, the muscle pairs were initially preshortened to about 75% of their standard length by previous stimuli, a method which has previously been used to measure activation heat[1]. The heat changes at 0 °C were followed with a Hill-type thermopile (90 chromel-constantan couples) connected to a sensitive galvanometer system. The light transmission was directly recorded in a cold room with the aid of a double-beam spectrophotometer, specially adapted for low-temperature measurements. In some experiments, in which the ATP content was additionally analyzed, the rephosphorylating reactions were inhibited by treating the muscles with 0.38 m*M* fluorodinitrobenzene according to Davies et al.[4]. This treatment had no effect on the heat and Ca^{2+} transients.

The results obtained for single stimuli (condenser discharges of 0.05 μF, 40 V) are presented in the Figure. If one compares the first time derivative of the activation heat with the kinetics of the Ca-murexide complex, it becomes obvious that, apart from the initial 25 msec, there exists a close relationship between the release and reabsorption of Ca^{2+} and the rate of heat production. To obtain some information as regards the changes in the level of ATP in the time course of activation, the muscles were frozen at 40 msec or at the end of the 370 msec period by immersing the experimental (as well as the unstimulated control) muscle rapidly in isopentane cooled with liquid nitrogen. As indicated by the voltage changes of the stimulus, later than 30 msec after the start of the freezing no longitudinal currents could pass along the muscle. Thus freezing must have been complete at 70 msec and 400 msec, respectively. Analyzing the ATP content of 32 muscle pairs fluorometrically, revealed that there was no detectable change in the ATP level which paralleled the

[1] A. V. Hill, Proc. R. Soc. B. *136*, 195 (1949).
[2] W. Hasselbach, Prog. Biophys. *14*, 167 (1964).
[3] F. F. Jöbsis and M. J. O'Connor, Biochem. biophys. Res. Commun. *25*, 246 (1966).
[4] R. E. Davies, M. J. Kushmerick and R. E. Larson, Nature *214*, 148 (1967).