These results indicate that the acute effect of GABA administered intraventricularly in cats is to cause sedation and catatonia, and that there is also a longer-lasting anticonvulsive effect which had been demonstrated in these experiments.

Summary. 1. The effects of intraventricular injections of tubocurarine, GABA and the interaction of these 2 drugs on the behavior of cats had been studied. 2. GABA delayed the onset and attenuated the effect of intraventricularly-injected tubocurarine. The ultimate effect of the combination of GABA and tubocurarine depended upon the drug which had been administered the week before. If it was GABA, no convulsions occurred. If it was tubocurarine, convulsions occurred only after a delay.

1. Elliott, K. A. C., Brit. Med. Bull., 1965, v21, 70.
2. Killam, K. F., Bain, J. A., J. Pharmacol., 1957, v119, 255.
3. Eidelberg, E., Baxter, C. S., Rabels, E., Saldias, C. A., Inhibition in the Nervous System and Gamma Aminobutyric Acid, Pergamon Press, New York, 1960.
4. Feldberg, W., Sherwood, S. L., J. Physiol., 1954, v123, 148.
5. Feldberg, W., Sherwood, S. L., ibid., 1953, v120, 3.
6. Merlis, J. K., Am. J. Physiol., 1940, v131, 67.
7. John, E. R., Killam, K. R., Wenzel, B. M., Tschirgi, R. D., Inhibition in the Nervous System and Gamma Aminobutyric Acid, Pergamon Press, New York, 1960.
8. Gammon, G. D., Gunmit, R., Kamrin, R. P., Kamrin, A., ibid., 1960.
9. Purpura, D. P., Girado, M., Smith, T. J., Gomez, J. A., Electroenceph. Clin. Neurophysiol., 1958, v10, 677.
10. Trivedi, C. P., Domer, F. R., Fed. Proc., 1965, v24, 516.
11. Purpura, D. P., Berl, S., Gonzalez-Monteagudo, O., Wyatt, A., Inhibition in the Nervous System and Gamma Aminobutyric Acid, Pergamon Press, New York, 1960.

Received January 7, 1966. P.S.E.B.M., 1966, v122.

Influence of Restricted Food Intake on Cardiac Glycogen Mobility.* (31076)

DON H. BLOUNT (Introduced by J. C. Stickney)
Department of Physiology, West Virginia University Medical Center, Morgantown

It has been known for some time that fasted animals produce elevated glycogen concentrations in the heart(5). More recently Adrouny and Russell demonstrated that when glycogen was separated into trichloroacetic acid-soluble (TCA) and residual fractions, the greatest change in the fasting animal occurred in the TCA fraction(1). These fractions have been so named because of the method used to isolate glycogen from the tissue. The tissue is homogenized in a trichloroacetic acid medium and then centrifuged. The glycogen in the resulting supernatant is called TCA glycogen and is assumed to be originally free from protein affiliation. The glycogen remaining in the protein residue is called residual glycogen and is thought to be bound to protein.

* This investigation was supported by USPHS Research Grant HE 06747.

Previous investigation has revealed that fasted and nonfasted animals display different degrees of glycogenolysis in TCA and residual fractions when forced to exercise by swimming (3,4). Experimentation in our laboratory has demonstrated that diet as well as fasting influences the glycogen levels of the heart (unpublished results). In the present experiment cardiac glycogen fractions were studied not only in fasted and nonfasted rats with a history of *ad libitum* feeding, but also in animals whose intake had been restricted so that their weights remained constant for 6 months. Animals with these dietary experiences were exercised by swimming so that cardiac glycogen mobility could be studied.

Methods and materials. The cardiac glycogen fractions (TCA and residual) were studied in 2 groups of animals: those on an *ad libitum* diet of Purina Laboratory Chow

TABLE I. Effect of Restricted Food Intake on Cardiac Glycogen Mobility.*

			No. of observations		Glycogen concentration	
			Rest	Swim	Rest	Swim
			Rats on normal diet			
A. Residual	nonfasted	l.v.	10	11	135 ± 6†	84 ± 9
		r.v.	8	9	136 ± 4†	87 ± 5
	fasted	l.v.	7	10	126 ± 8	106 ± 11
		r.v.	9	8	139 ± 7	122 ± 16
B. TCA	nonfasted	l.v.	10	11	74 ± 6†	42 ± 4
		r.v.	9	10	93 ± 10†	34 ± 4
	fasted	l.v.	8	10	173 ± 12‡	107 ± 14
		r.v.	9	8	163 ± 13	117 ± 18
			Rats on restricted diet			
A. Residual		l.v.	12	10	78 ± 14	96 ± 14
		r.v.	11	10	88 ± 10	84 ± 11
B. TCA		l.v.	12	11	46 ± 10	52 ± 9
		r.v.	11	11	64 ± 18	62 ± 11
			Rats on restricted diet in fasted condition			
A. Residual		l.v.	2	2	207	201
		r.v.	2	2	113	138
B. TCA		l.v.	2	2	314	298
		r.v.	2	2	300	317

* Left ventricle (l.v.) and right ventricle (r.v.) values given as mg/100 g wet tissue; mean ± S.E.

† $P < .001$. ‡ $P < .005$.

(divided into 24-hour fasted and nonfasted categories), and those animals placed on a restricted intake of Purina Laboratory Chow for a 6-month period. An additional 4 rats were taken from the group on restricted intake and their glycogen fractions studied after a 48-hour fast. Within each group rats were exercised by swimming in water at 25°C for 15 minutes and the cardiac glycogen fractions compared with unexercised animals. Body weights of the restricted intake group were maintained at an average value of 286 g, and the rats were approximately 9 months old at the time of sacrifice. The 24-hour fasted rats and the nonfasted rats were 7 and 11 months old respectively at time of sacrifice.

Immediately after the exercise period the animals were decapitated, the heart removed, and the atria and aorta discarded. The ventricles were then separated into right ventricular wall and left ventricular wall with interventricular septum attached. The right ventricle was then frozen between cakes of dry ice, and the left ventricle with attached interventricular septum was weighed and homogenized in a Ten-Broeck tissue grinder with cold 10% trichloroacetic acid. This process required approximately 2½ minutes from time of sacrifice. The right ventricle was then weighed and homogenized. This technic of handling the ventricular tissue does not affect the pattern of glycogenolysis as seen in previous studies in this laboratory (unpublished data). The glycogen was separated into TCA and residual fractions according to the method established by Bloom *et al*(2), and both glycogen fractions were analyzed by the anthrone method of Seifter *et al*(6).

The glycogen values are reported in milligrams of glycogen per 100 g of wet tissue. The statistical analyses were made using the Student *t* test of significance.

Results. The nonfasted rats with a history of *ad libitum* feeding demonstrated glycogenolysis in the residual and TCA fractions of both left and right ventricles as the result of swimming (Table I). The only glycogenolysis seen in the fasted animals of this group was in the TCA fraction of the left ventricle. These results are in direct contrast to those found in animals with the history of restricted intake (also shown in Table I). No glycogenolysis was seen in either glycogen fraction for left or right ventricles of the swimming rats, and the TCA fractions in this population were considerably lower than the residual

fractions. All but one of the fractions of the unexercised animals with the restricted intake were significantly lower than the fractions of the nonfasted, unexercised animals fed *ad libitum* ($P<0.04$, with the exception of the right ventricular TCA value).

In an effort to determine whether or not lack of glycogen mobilization was due to the limited quantities of glycogen present, 4 animals from the group with restricted intake were fasted approximately 48 hours, and 2 animals from this group were forced to swim for the 15-minute period. The other 2 were sacrificed in the rested condition and compared with the exercised animals. Again there was no indication of glycogenolysis in the swimming animals even though the glycogen concentrations were the highest seen in any of the rats studied.

Discussion. Glycogenolysis was shared more equally in both residual and TCA fractions of nonfasted animals, whereas the cardiac glycogenolysis which occurred in fasted animals was limited to the TCA fraction (in the left ventricle). The data suggest that TCA glycogen was preferred over residual glycogen for energy in the fasted animal subjected to exercise. In the nonfasted rat less glycogen was available in the TCA fraction, and cardiac tissue was forced to rely more heavily on residual glycogen. In the TCA fractions of fasted animals, previously fed *ad libitum,* significant glycogenolysis is seen in the left ventricle, whereas the right ventricle displays a reduced, but nonsignificant, glycogen concentration in the swimming rats. This general pattern of glycogenolysis seen in the present groups of fasted and nonfasted rats is also typical of animals that are considerably younger(3,4).

The animals with the restricted intake demonstrated no change in glycogen reserves of the heart when forced to exercise. This did not appear to hamper their ability to swim. The lack of glycogenolysis seen in animals with restricted intake is apparently not due to the somewhat limited concentration available at the beginning of the exercise. Regardless of how low initial concentrations of myocardial glycogen have been in animals other than those on restricted intake, swimming has always produced glycogenolysis in our laboratory. Since a limited number of 48-hour fasted animals previously on a restricted intake was used in this experiment, conclusions may be drawn only with caution. However, it appeared that these 48-hour fasted animals displayed elevated cardiac glycogen fractions that were not utilized by the heart tissue in these acute experiments. This suggests that lack of glycogenolysis in animals on restricted intake was not a function of initial concentration.

The mechanism operating within the myocardium of animals with a history of restricted food intake remains obscure. Whatever the mechanism, it is apparent that by limiting food intake one does not find the glycogenolysis usually seen in exercising rats. This lack of glycogenolysis does not appear to be due to the initially lower quantity of cardiac glycogen present in the animals on restricted intake.

Summary. Cardiac glycogen mobility was compared in swimming rats that had a history of *ad libitum* feeding with rats placed on a restricted intake for 6 months. Swimming, nonfasted rats (fed *ad libitum*) demonstrated cardiac glycogenolysis in both residual and TCA fractions, whereas fasted animals in this group exhibited significant glycogenolysis in the TCA fraction of the left ventricle only. Rats with the restricted intake demonstrated no glycogenolysis in either glycogen fraction of the right or left ventricle after the swimming period. This lack of glycogenolysis did not appear to be due to somewhat limited quantities of glycogen available in the animals on restricted intake.

1. Adrouny, G. A., Russell, J. A., Endocrinol., 1956, v59, 241.

2. Bloom, W. L., Lewis, G. T., Schumpert, M. Z., Shen, T., J. Biol. Chem., 1951, v188, 631.

3. Blount, D. H., Meyer, D. K., Am. J. Physiol., 1959, v197, 1013.

4. Blount, D. H., Stickney, J. C., Fed. Proc., 1964, v23, 958.

5. Evans, G., J. Physiol., 1934, v82, 468.

6. Seifter, S., Dayton, S., Novic, B., Muntwyler, E., Arch. Biochem., 1950, v25, 191.

Received January 7, 1966. P.S.E.B.M., 1966, v122.