TABLE I. "Free" Tryptophan Residues of Native α_1-Acid Glycoprotein in Different Solvents.

2-Chloroethanol, %	No. of residues per mole of protein	Specific optical rotation $[\alpha]^{23}_{516}$
0	1.9	—26°
20	2.5	—43°
50	3.7	—24°

number of "free" tryptophyl residues was calculated from these measurements.

The results obtained are summarized in Table I. In aqueous solution 2 of the 4 tryptophyls of native α_1-acid glycoprotein were found to be "free." In the presence of 50% chloroethanol, the remaining 2 tryptophan residues also reacted with this reagent. Essentially identical results were obtained when the sialic acid-free form of this glycoprotein was investigated. This study further suggests that the carbohydrate moiety does not interfere with these determinations on glycoproteins. Optical rotation measurements of α_1-acid glycoprotein also demonstrated the changes in the structure of this protein in presence of chloroethanol.

In the second part of this study, the "free" tyrosine residues of α_1-acid glycoprotein were determined. The method of Vallee and coworkers(9) involving the reaction with acetylimidazole at pH 7.5 was used. In aqueous solution 5 of the 12 residues(10) reacted with this reagent. In presence of 8 M urea without change in pH essentially all residues were found to be "free." The optical rotation of α_1-acid glycoprotein in 8 M urea was reported earlier to be increased to 64° in a reversible fashion(11).

Summary. The "free" tryptophan and tyrosine residues of α_1-acid glycoprotein (orosomucoid) were determined with the highly specific reagents of 2-hydroxy-5-nitrobenzylbromide and acetylimidazole, respectively. In the native protein 2 of the 4 tryptophyls and 5 of the 12 tyrosyls were "free." In the presence of 50% 2-chloroethanol or 8 M urea, essentially all these residues reacted with the reagents mentioned.

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Received July 18, 1966. P.S.E.B.M., 1967, v124.

Inhibitory Effect of Chlorothiazide *in vitro* on Glucose Metabolism of Adipose Tissue.* (31707)

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Chlorothiazide added directly to the incubating medium reduces the *in vitro* rate of utilization of glucose by epididymal fat pads of rats(1). This inhibitory action of chlorothiazide was demonstrated with high concentrations of the drug. The effect *in vitro* of lower chlorothiazide concentrations, therefore, is presented here.

Methods. Six experiments were carried out in which the rate of utilization of glucose

* Supported by grants from Michigan Heart Assn. and USPHS HE-08083-03 from NIH.

in vitro by normal rats' epididymal fat pads was measured in the presence of various chlorothiazide concentrations in the incubating medium. In all experiments non-fasting male Sprague-Dawley rats weighing 220-260 g were used. Rats were decapitated, epididymal fat pads removed and each peripheral 5 mm of fat pad was cut into 8 pieces in buffer solution at 37°C. Four pieces of fat pad, each from a different rat, were placed into a tared stoppered flask containing 3 ml chilled modified Krebs bicarbonate buffer solution containing 200 mg glucose/100 ml and previously equilibrated with 5% CO_2-95% O_2 at room temperature for 5 minutes. Media were prepared with 0, 0.001, 0.01, 0.1 or 1 mM chlorothiazide/liter and, in some cases, with 0.25 unit crystalline insulin† and 2 mg gelatin/ml. Flasks were shaken for 2 hours at 37°C, then flasks plus tissues were weighed. Media glucose determinations before and after incubation were done by the Nelson-Somogyi method(2).

Results. In all experiments as the chlorothiazide concentration in the incubating medium was increased there was a decrease in the glucose disappearance rate from the medium (Table I). When chlorothiazide was added to the incubating medium in a concentration of 1×10^{-3} molar, the mean rate of decrease of the glucose concentration of the medium for all experiments was diminished to 44% of the normal rate (Fig. 1). This reduction in rate of utilization of glucose occurred whether or not insulin was present in the medium. The large variability between experiments, due to the fact that they were carried out at different times with fat pads from different groups of animals, obscured the stimulatory effect of insulin on glucose utilization.

Discussion. Benzothiadiazine drugs alter carbohydrate metabolism through multiple mechanisms. One of these is to reduce the level of serum insulin-like activity(3). Such reduction will decrease the rate of entry of glucose into cells where this is facilitated by insulin. The lower level of serum insulin-like activity also brings about a decrease in the

† Kindly supplied by Eli Lilly and Co.

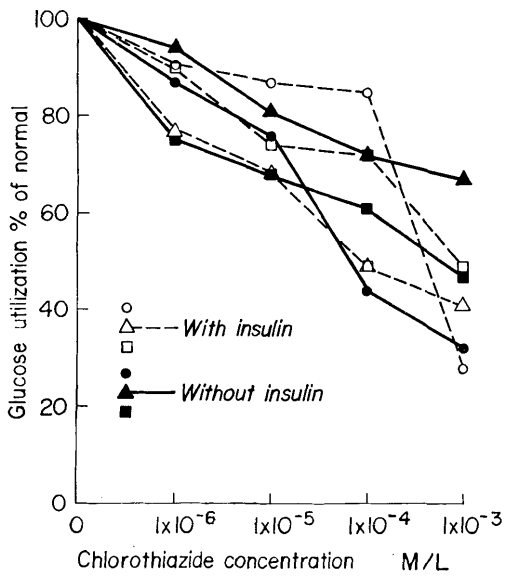

FIG. 1. The mean rate of utilization of glucose by adipose tissue *in vitro,* expressed as per cent of the rate when no chlorothiazide is present in the medium is plotted for each of the 6 experiments as a function of the molar concentration of chlorothiazide in the medium.

activities of liver glucokinase and dihydroxyacetonekinase(4), as the synthesis of these enzymes requires insulin. Still another action of the benzothiazide drugs, however, is a direct one on adipose tissue as demonstrated here. This effect is not dependent on lowering of the serum insulin-like activity. Indeed, the *in vitro* rate of glucose utilization by normal rats' epididymal fat is reduced by chlorothiazide whether insulin is, or is not, added to the medium. The decrease in rate of utilization of glucose by adipose tissue due to the presence of chlorothiazide is evident even when the concentration of this drug is as low as 1×10^{-6} molar ($P<0.01>0.001$ for the mean of all experiments with insulin in the medium and 0.05 for the mean of all experiments without insulin.) This inhibition by low concentrations of chlorothiazide suggests that a metabolic step is impaired which is quite sensitive to this agent. The fact that the mean rate of glucose utilization is decreased 56%, not 100%, by relatively high concentrations of chlorothiazide (1×10^{-3} molar) may be due to alternate pathways for glucose metabolism not being inhibited by this drug.

TABLE I. Rate of Glucose Utilization of Rats' Adipose Tissue with Varying Concentrations of Chlorothiazide in the Medium.

Exp No.	Chlorothiazide concentration (M/l) 1×10^{-3}	1×10^{-4}	1×10^{-5}	1×10^{-6}	0
			With insulin		
I	2.35	3.57	4.04	6.12	8.86
	1.00	4.75	5.06	6.65	4.26
	.83	7.15	7.04	5.46	3.00
	3.77	6.86	8.05	4.00	4.80
	2.62	4.20	5.70	7.54	6.59
	1.42	6.24	8.38	6.32	8.59
	1.71	7.86	4.32	5.89	9.85
	1.55	5.34	4.74	6.97	8.19
Mean ± S.D.	1.91 ± .91	5.74 ± 1.42	5.92 ± 1.59	6.13 ± 1.00	6.77 ± 2.33
II	5.06	6.81	3.92	3.96	8.15
	2.84	4.51	1.01	6.42	4.94
	4.20	7.63	3.21	3.89	6.57
	.71	4.49	3.23	5.24	6.00
	1.96	2.66	6.41	5.16	2.42
	2.22	2.24	7.24	3.62	7.75
	4.65	2.19	5.42	7.08	4.88
	.98	2.64	4.07	6.43	5.60
Mean ± S.D.	2.83 ± 1.55	4.15 ± 1.98	4.31 ± 1.86	5.23 ± 1.24	5.79 ± 1.36
III	4.09	5.30	5.14	7.77	6.44
	3.66	5.00	5.73	3.47	9.58
	3.49	4.10	5.33	6.71	6.74
	2.22	2.15	7.53	7.46	9.19
	2.10	2.32	8.08	7.52	8.08
	6.43	3.10	4.29	5.19	8.16
	3.68	—	—	9.61	11.82
	4.10	—	—	—	11.23
Mean ± S.D.	3.72 ± 1.25	3.66 ± 1.23	6.02 ± 1.34	6.82 ± 1.83	8.91 ± 1.82
			Without insulin		
IV	3.58	2.03	—	5.89	10.33
	2.80	4.94	6.74	5.00	11.19
	5.00	6.27	7.20	6.76	13.44
	2.26	4.82	6.87	6.94	5.70
	3.53	4.70	8.05	12.85	7.10
	2.93	3.08	7.49	11.50	6.80
	2.57	3.51	7.32	14.80	—
	2.10	5.14	7.98	5.11	13.28
Mean ± S.D.	3.10 ± .86	4.31 ± 1.26	7.38 ± .47	8.61 ± 3.60	9.69 ± 2.94
V	5.38	5.01	4.94	4.00	6.48
	—	—	2.30	6.97	7.20
	3.79	5.50	2.86	5.48	6.44
	1.00	3.50	6.57	6.06	7.82
	5.86	5.00	6.58	—	5.89
	3.73	4.05	—	5.28	9.43
	3.02	—	5.12	5.61	8.71
	1.94	—	6.64	—	7.83
Mean ± S.D.	3.53 ± 1.61	4.61 ± .73	5.00 ± 1.67	5.57 ± .84	7.48 ± 1.13
VI	4.04	6.16	6.96	6.35	6.76
	3.61	8.44	4.50	8.67	9.93
	5.76	6.22	6.21	7.57	10.68
	5.99	5.70	8.44	4.80	8.74
	5.48	2.66	7.72	9.02	4.41
	3.79	4.16	6.97	—	6.48
	6.67	5.00	4.30	6.01	6.25
	6.58	6.77	5.38	8.68	9.15
Mean ± S.D.	5.24 ± 1.17	5.64 ± 1.63	6.31 ± 1.40	7.30 ± 1.49	7.80 ± 2.00

Values expressed as mg glucose utilized per g wet tissue per 2 hr.

A peripheral action of the benzothiadiazine compounds on tissue glucose metabolism has been suggested by *in vivo* experiments. Tabachnick *et al* found that the hyperglycemia due to diazoxide occurs even in depancreatized or alloxanized animals(5). Evidence for a possible peripheral effect of chlorothiazide on membrane premeability to phosphorus has been presented by Beardwood *et al*(6). The current studies substantiate a direct action of these agents on fat tissue.

Summary. Chlorothiazide added directly to the incubating medium decreases the *in vitro* rate of utilization of glucose by rats' adipose tissue. The degree of reduction in glucose utilization is greater as the amount of chlorothiazide present in the medium is increased. It can be demonstrated even with low concentrations of the drug (1×10^{-6} molar). These experiments demonstrate a direct inhibitory effect of chlorothiazide on the glucose utilization of fat tissue.

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Received July 28, 1966, P.S.E.B.M., 1967, v124.

Brain Maturation in the Neonatal Rat after Varied Light Cycles.* (31708)

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Previous studies have shown effects of varied light cycles upon growth and reproduction of birds and mammals(1,2,3). Continuous illumination directed on specific hypothalamic areas influenced gonadotropin release and the estrous cycle(4,5). LH concentration was markedly decreased in the pituitaries of rats after 50 days of continuous light(6). Furthermore, photovoltaic cells stereotaxically implanted in the hypothalamus of rodents have shown that light penetrates the mammalian brain(7).

This investigation was undertaken to study the effects of various light patterns upon brain maturation and brain convulsibility. The rat was the experimental animal used because its central nervous system is still partially immature during the first few weeks of life(8).

* Supported by NIH Grant NB-05272 and NB-06669.

† Part of this work was done during the author's tenure at New York Medical College, Dept. of Anatomy.

The phases of postnatal brain development in the rat are characterized by specific responses to electroshock and a correlation exists between these responses and developmental changes which are both biochemical and structural(9,10).

Material and methods. A total of 16 litters, 8 Long-Evans rats per litter, was used for this experiment. A standard rat pellet diet and water were given *ad libitum* to the mothers and after weaning to the young rats. The animal rooms had a constant temperature of 72°F and were artificially illuminated by automatic light switches regulated for control and experimental groups.

Beginning at one day of age 4 litters with mothers were subjected to one of the following light schedules: 1) 12 hours of light followed by 12 hours of dark, control environment, 2) 6 hours of light alternated by 6 hours of dark, 3) continuous light and 4) continuous dark.

Convulsive seizures were produced by electroshock stimulation through corneal elec-