homogenized in 9 vol. of cold 0·25 M sucrose and the mitochondria isolated by differential centrifugation, washed four times, and made into acetone powders. Extracts of the latter were made and were assayed for glycine acyltransferase as described by Schachter and Taggart;[1] this consisted of measuring the decrease in absorbance at 280 mμ as the thioester bond of benzoyl-SCoA is cleaved. Protein was determined by the biuret reaction.

RESULTS

The enzyme activities of material prepared from animals of different ages are shown in Fig. 1. All points represent the results of six experiments except that five experiments were performed on 30-day-old. animals, and four experiments were performed on 19-day fetuses and on 90-day-old animals. There is relatively little activity in fetal and early newborn life, and by the time of weaning (20 days) an appreciable increase has taken place. This proceeds to a maximum at 30 days of age then decreases to a small extent. This development pattern is similar to that described for hippuric acid synthesis by liver homogenates,[4] suggesting that glycine acyltransferase may be the rate-limiting step in the overall reaction. These experiments further define the probable biochemical basis for the reduced ability of newborn infants to metabolize *p*-aminobenzoic acid.[5]

Acknowledgements—The competent technical assistance of Margaret P. Holbrook is gratefully acknowledged. Supported by a grant from the United States Public Health Service (GM-06992).

Department of Pediatrics,
Yale University School of Medicine,
New Haven, Conn, U.S.A.

IRA K. BRANDT

REFERENCES

1. D. SCHACHTER and J. V. TAGGART, *J. biol. Chem.* **208**, 263 (1954).
2. M. DIXON and E. C. WEBB, *Enzymes*, 2d ed., p. 706. Academic Press, New York (1964).
3. R. K. KIELLEY and W. C. SCHNEIDER, *J. biol. Chem.* **185** 869 (1950),
4. I. K. BRANDT, *Devl. Biol.* **10**, 202 (1964).
5. M. F. VEST and R. SALZBERG, *Archs Dis. Childh.* **40**, 97 (1965).

Biochemical Pharmacology, 1966, Vol. 15, pp. 995–998. Pergamon Press Ltd., Printed in Great Britain.

The effect of carbaryl (1-naphthyl-N-methyl carbamate) on blood glucose, and liver and muscle glycogen in fasted and nonfasted rats

(*Received* 5 *November* 1965; *accepted* 2 *March* 1966)

WEISS *et al.*[1] have reported that intraperitoneal administration of toxic doses of carbaryl (1-naphthyl-N-methyl carbamate) produced a pronounced hyperglycemic response in nonfasted rats and that this response was absent in adrenalectomized rats.[2] This suggests that the hyperglycemia induced by carbaryl may be due to the release of epinephrine from the adrenal gland.

Fleming and Kenny[3] reported that subcutaneous injections of several catechol amines produced hyperglycemia and glycogenolysis in skeletal muscle of both fasted and nonfasted rats. Although the blood glucose rise was lower in fasted rats, the glycogenolytic response in the muscle was independent of the prandial state of the animals.

The purpose of the present study was to determine what effect the release of endogenous catecholamines by carbaryl would have on blood glucose and liver and muscle glycogen of fasted and nonfasted rats. The two doses of carbaryl chosen were 5 mg/kg, which produced minimal, if any, cholinergic effects, and 25 mg/kg, which caused pronounced signs of cholinergic stimulation.

MATERIALS AND METHODS

Carbaryl of 99·5% purity (Union Carbide Chemical Corp.) was dissolved in dimethyl formamide (Fisher Scientific Co.) and injected intraperitoneally in doses of 5 and 25 mg/kg in volumes of 1 ml/kg of body weight.

Male rats (200–300 g) of the Osborne–Mendel strain, bred in the FDA laboratories, were maintained on a diet of Purina laboratory chow and had water available *ad libitum*. Fasted rats received no food for 24 hr prior to the start of the experiment.

The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and decapitated 10 min later. Glucose was determined on whole blood by the method of Hoffman[4] with the use of an Auto-Analyzer (Technicon Instrument Corp.).

The liver and right gastrocnemius muscle were each removed from the anesthetized animal and were then weighed and quickly frozen in a container immersed in dry ice and acetone. In order to eliminate any errors due to sampling, the entire liver and muscle were used. Glycogen was estimated by the anthrone method of Seifter *et al.*[5]

RESULTS

Blood glucose. After injection of 5 and 25 mg carbaryl/kg to both fasted and nonfasted rats (Table 1), the blood glucose rose in 15 min, and a further significant rise occurred between 15 and 30 min after injection. In addition, the elevated blood glucose levels were maintained for 60 min. After injection of the 25 mg/kg dose, the blood glucose rise was usually greater than that occurring after

TABLE 1. BLOOD GLUCOSE* AND LIVER AND MUSCLE GLYCOGEN† AFTER INTRAPERITONEAL ADMINISTRATION OF CARBARYL (5 AND 25 mg/kg) TO FASTED AND NONFASTED RATS

	Controls‡	15 min	30 min	60 min
		(5 mg/kg injected)		
Blood glucose				
Fasted	66 ± 5	76 ± 6	99 ± 11§	102 ± 6§
Nonfasted	98 ± 2	112 ± 3§	137 ± 9§	135 ± 13§
Liver glycogen				
Fasted	144 ± 16	114 ± 23	146 ± 24	149 ± 23
Nonfasted	3515 ± 264	3544 ± 382	3149 ± 340	2735 ± 352
Muscle glycogen				
Fasted	188 ± 19	204 ± 14	175 ± 15	176 ± 18
Nonfasted	363 ± 22	285 ± 25§	242 ± 43§	257 ± 25§
		(25 mg/kg injected)		
Blood glucose				
Fasted	79 ± 3	109 ± 14§	99 ± 6§	150 ± 14§
Nonfasted	100 ± 3	158 ± 4§	189 ± 9§	209 ± 3§
Liver glycogen				
Fasted	173 ± 10	134 ± 14	115 ± 28	172 ± 26
Nonfasted	3655 ± 398	3402 ± 312	2810 ± 298	2837 ± 366
Muscle glycogen				
Fasted	202 ± 37	138 ± 19	153 ± 22	124 ± 21
Nonfasted	334 ± 29	252 ± 18§	204 ± 22§	220 ± 23§

* Expressed as mg glucose/100 ml whole blood.

† Expressed as mg glycogen (glucose equivalent)/100 g wet tissue.

‡ Composite of values obtained at 15, 30, and 60 min after injection of dimethyl formamide.

§ Figures are significantly different from control, $P < 0.05$. Student's t-test was used in the statistical evaluation of the results.
Each figure represents mean ± S.E. of 10–15 animals.

injection of the lower dose of carbaryl. The blood glucose increases after carbaryl in fasted and nonfasted rats, while smaller in the former, were generally proportional to the respective pretreatment levels.

Liver and muscle glycogen. The concentration of liver glycogen in fasted control rats fell sharply to about 5% of the normal nonfasted level. There was no significant alteration in liver glycogen in either fasted or nonfasted rats after administration of 5 or 25 mg carbaryl/kg.

The muscle glycogen of fasted control rats was approximately 50 per cent of the nonfasted level. In nonfasted rats, a significant decrease in muscle glycogen was observed at 15 min after injection of carbaryl at 5 and 25 mg/kg, and this decreased level persisted for 60 min after treatment. Since carbaryl-induced brain cholinesterase inhibition and increases in blood glucose reached maximal values 15–60 min after injection,* our observations were not extended beyond this time interval. There was no significant alteration in muscle glycogen content of fasted rats at either dose of carbaryl.

Cholinergic effects. After administration of 5 mg carbaryl/kg, no tremors or other signs of cholinergic stimulation resulting from the anticholinesterase activity of carbaryl were observed. After injection of 25 mg/kg, rats showed marked tremors, which became apparent within 10 min and increased in intensity to a maximum at 30 min. The severity of the tremors then began to subside, although they were still apparent 60 min after injection. Pronounced lacrimation, salivation, defecation, and micturition were also observed.

Rat brain cholinesterase activity. The following results, obtained by manometric assay of brains from another group of nonfasted rats, are included for the purpose of completeness. After administration of 5 mg carbaryl/kg, rat brain cholinesterase activities at 15, 30, and 60 min were 75%, 84%, and 84% of control values respectively. At corresponding time intervals, brain cholinesterase activity was 49%, 36%, and 54% of the controls after the injection of 25 mg carbaryl/kg.

DISCUSSION

From the effects noted, a correlation seems to exist between the onset and duration of tremors, the occurrence and extent of the rise in blood glucose, and the degree of brain cholinesterase inhibition following the administration of carbaryl.

In rats treated with 25 mg carbaryl/kg, the hyperglycemic response has been shown by Weiss *et al.*[2] to be completely blocked by adrenalectomy and unaltered by hypophysectomy. A significant increase in blood glucose is not known to occur in acute or chronically stressed animals from the release of ACTH.† Therefore, the blood glucose effects do not seem to be due to a common stress response which involves the adrenal gland and the hypophysis. It is possible that the hyperglycemia induced by carbaryl may be due to, or accompanies, inhibition of cholinesterase in the adrenal medulla, thereby causing secretion of epinephrine. This catecholamine is known to cause hyperglycemia and glycogenolysis in liver and skeletal muscle.

In our experiments, we obtained significant hyperglycemic responses in both fasted and nonfasted rats and also significant decreases in muscle glycogen levels of nonfasted rats after administration of doses of carbaryl of 5 and 25 mg/kg. Liver glycogen content was not significantly altered under the conditions of this experiment, and one might speculate that the variation in liver glycogen levels was too great to reflect changes in blood glucose levels.

The hepatic glycogenolytic response was not significantly altered by fasting, and it appears that the actual level of liver glycogen did not contribute to the onset or duration of the hyperglycemic response at the various time intervals after injection of similar doses of carbaryl to fasted and nonfasted rats.

The failure to obtain a decrease in muscle glycogen levels of fasted rats and an alteration in liver glycogen content of fasted and nonfasted rats remains unexplained.

Division of Toxicological Evaluation,
Bureau of Scientific Standards and Evaluation,
Food and Drug Administration,
U.S. Dept. of Health, Education and Welfare,
Washington, D.C., U.S.A.

R. A. ORZEL
L. R. WEISS

* Unpublished results from this laboratory.
† Dwight J. Ingle, personal communication.

REFERENCES

1. L. R. WEISS, J. BRYANT and O. G. FITZHUGH, *Toxicol. appl. Pharmac.* **6**, 363 (1964).
2. L. R. WEISS, R. A. ORZEL and O. G. FITZHUGH, *Fedn. Proc.* **24**, 641 (1965).
3. W. W. FLEMING and A. D. KENNY, *Br. J. Pharmac.* **22**, 267 (1964).
4. W. S. HOFFMAN, *J. biol. Chem.* **120**, 51 (1937).
5. S. SEIFTER, S. DAYTON, B. NOVIC and E. MUNTWYLER, *Archs Biochem.* **25**, 191 (1950).

Biochemical Pharmacology, 1966, Vol. 15, pp. 998–1000. Pergamon Press Ltd., Printed in Great Britain.

The influence of phenazinium upon glycolysis and the pentose pathway in human red cells*

(*Received* 27 *November* 1965; *accepted* 2 *March* 1966)

BIOLOGICAL interest in phenazines began with the discovery of the bacterial pigment pyocyanine[1–3] and with its suppressive effects on the growth of a variety of bacteria.[4–7] That some phenazines directly affect growth processes in metazoa,[8–11] including tumor growth[12, 14] has led recently to examination of a number of these substances as potential antitumor agents.[14, 15] One of these, 1:3-diamino-5-, methyl phenazinium chloride (phenazinium,† Fig. 1), has some activity against mouse leukemia 1210, and is at present undergoing clinical trial in human neoplasia. Its metabolic effects are being currently studied, and preliminary data upon glycolysis and the pentose pathway, with the human red cell as a model system, are here presented.

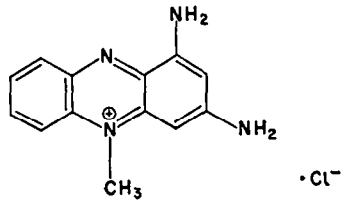

FIG. 1. Phenazinium.

EXPERIMENTAL

Studies were carried out in a standard Warburg apparatus at 37°, the period of incubation being 3 hr. The incubation medium was 0·15 M phosphate buffer, pH 7·7, 0·08 M in glucose. All determinations were performed in triplicate and the mean used as the final result. Oxygen consumption was determined manometrically, CO_2 production by the recovery of $^{14}CO_2$ obtained from substrate glucose labeled with ^{14}C in the 1-, 2-, 6-positions, and from universally labeled glucose, the counts being measured in a Packard Tri-carb scintillation counter by the method of Passmann *et al.*[16] Lactate production was studied by the methods of Barker and Summerson[17] and by a lactate dehydrogenase

* This work was supported by Public Health Service Research Grant CA 06939 from the National Cancer Institute, Bethesda, Md.

† Phenazinium supplied by the Clinical Branch, Collaborative Research, National Cancer Institute, U.S. Public Health Service, through the Southwest Cancer Chemotherapy Study Group.