was extremely small and during incubation of cortex tissue in an eserine sulphate medium containing 25 mM KCl with or without atropine the ACh concentration of the tissue fell to about 4 μg/ml. In subsequent experiments on soman treated brain cortex slices it appeared that eserine sulphate (0·4 mM) strongly inhibits the uptake of ACh. This explains why Elliott and Henderson[9] who 15 years ago demonstrated uptake of ACh into brain slices in an eserine sulphate medium, found only a comparatively small effect.

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REFERENCES

1. J. F. Mitchell, *J. Physiol.* **165** (1963) 98.
2. J. C. Szerb, *Canad. J. Physiol. Pharmac.* **42** (1964) 303.
3. R. L. Polak, *J. Physiol.* **181** (1965) 317.
4. N. J. Giarman and Pepeu, *Br. J. Pharmac.* **23** (1964) 123.
5. K. A. C. Elliott, R. L. Swank and N. Henderson, *Am. J. Physiol.* **162** (1950) 469.
6. W. Feldberg, *J. Physiol.* **103** (1945) 367.
7. P. J. G. Mann, M. Tennenbaum and J. H. Quastel, *Biochem. J.* **33** (1939) 822.
8. R. Creese and D. B. Taylor, *Life Sci.* **4** (1965) 1545.
9. K. A. C. Elliott and N. Henderson, *Am. J. Physiol.* **165** (1951) 365.

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Carbamylaspartate, a new agent against acute ammonia intoxication*

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INTRODUCTION

In recent years studies have been carried out in our Laboratory on the mechanism of acute ammonia intoxication[1] in which some amino acids showed a distinct protective effect.[2,3] Ornithine-aspartate mixture was able to suppress in the rat the toxic effects of a LD_{50} of ammonium acetate when injected intraperitoneally. L-aspartate alone was almost inactive; it became very active when associated with L-ornithine which by itself showed already much activity. The mechanism of this protection was consistent with an enhancement of the Krebs-Henseleit urea cycle.[4] L-arginine protected even better rats intoxicated with ammonia;[3] however, its mechanism of protection cannot be entirely explained on the basis of an enhancement of Krebs-Henseleit urea cycle.[5]

The present paper deals with the effect of carbamylaspartate (CA)† on acute ammonia intoxication. CA is an intermediate compound in the pathway leading to pyrimidine nucleotides.[6] Its role in urea synthesis has been recently discussed.[7,8]

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† The following abbreviations are used in this paper: CA—carbamylaspartate; AA—ammonium acetate.

EXPERIMENTAL

Male Wistar albino rats weighing 150–180 g were used for these experiments. The animals, fed on normal laboratory chow, were fasting, except for water *ad lib.*, for 12–14 hr prior to study.

D, L-carbamylaspartic acid was tested in three doses each in the same volume of aqueous sodium hydroxide solutions adjusted to pH 7·0 so that all the rats received the same volume of 10 ml/kg body weight. CA was then given at doses of 2·0, 3·0 and 4·0 m-moles/kg body weight in three different groups of animals. A fourth group acting as controls, was given 10 ml/kg body weight of saline.

One hour after CA or saline administration, the animals were treated with ammonium acetate (AA) solution (10 ml/kg) with a dose of 8·5 m-moles/kg body wt. (LD_{50}).

All the above solutions were given intraperitoneally.

The animals were observed for 12 hr after intoxication during which mortality and convulsions were taken in account.

RESULTS AND DISCUSSION

Table 1 summarizes results of protective effect of CA administered at various doses 1 hr prior to ammonia intoxication. CA at doses of 3·0 and 4·0/kg body weight shows a definite protective effect

TABLE 1. PROTECTIVE EFFECT OF CARBAMYLASPARTATE (CA) IN RATS INTOXICATED WITH LD_{50} (8·5 m-moles/kg) OF AMMONIUM ACETATE (AA)

Treatment	No. of animals	Mortality (%)	Convulsions (%)
NaCl + AA	265	53·2	89·4
CA (4·0 m-moles/kg) + AA	152	7·9	21·7
CA (3·0 m-moles/kg) + AA	109	10·1	27·4
CA (2·0 m-moles/kg) + AA	90	27·7	44·4

with slight difference between the two mentioned doses. Whereas 2·0 m-moles/kg of CA show a much less protective effect as indicated in Table 1. We also observed that the number of convulsions showed by each control rat was always higher than the number of convulsions showed by those treated animals which underwent convulsions.

Experiments devoted to establish the optimal time at which CA administration protects from ammonia intoxication indicated this be 1 hr prior to intoxication.

In conclusion, data herein reported indicate that CA is able to prevent ammonia intoxication to a considerable extent.

At the present time we cannot explain the mechanism through which CA protects from ammonia intoxication and experiments are in progress on this subject.

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REFERENCES

1. F. SALVATORE, V. BOCCHINI and F. CIMINO, *Biochem. Pharmac.* **12**, 1 (1963).
2. F. SALVATORE and V. BOCCHINI, *Nature, Lond.* **191**, 705 (1961).
3. V. BOCCHINI and F. SALVATORE, *Ital. J. Biochem.* **10**, 483 (1961).
4. D. CITTADINI, F. CIMINO, MARIA D'AYELLO CARACCIOLO and F. SALVATORE, *Life Sci.* **3**, 61 (1964).
5. F. SALVATORE, F. CIMINO, MARIA D'AYELLO CARACCIOLO and D. CITTADINI, *Archs Biochem. Biophys.* **107**, 499 (1964).
6. I. LIEBERMAN and A. KORNBERG, *J. biol. Chem.* **207**, 911 (1954).

7. G. Della Pietra, E. Rogliani, S. Procaccini and C. Rogliani, *Archo Sci. biol.* **42**, 523 (1958).
8. R. Crokaert and J. P. Baroen, *Bull. Soc. Chim. biol.* **47**, 687 (1965).
9. J. P. Greenstein, M. Winitz, P. Gullino, S. M. Birnbaum and M. C. Otey, *Archs Biochem. Biophys.* **64**, 342 (1956).

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Glycine acyltransferase activity in developing rat liver

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The detoxication of benzoic acid and similar substances in mammalian tissues is brought about in three stages.[1] Benzoyl adenylate is formed by pyrophosphate exchange with ATP. The adenylate moiety is then exchanged for coenzyme A to produce benzoyl-CoA. Finally, the latter reacts with glycine to give hippuric acid with the regeneration of free CoA. The first two reactions are catalyzed by a thiokinase, the last by glycine acyltransferase (E.C. 2.3.1.13[2]), the specificity of which is indicated by its systematic name: acyl-CoA: glycine N-acyltransferase. The three activities are found only in the mitochondrial fraction of liver and kidney.[3]

Hippuric acid synthesis by liver homogenates has been previously shown to vary with the age of the animal.[4] The present study demonstrates that the activity of the glycine acyltransferase component of the system also varies with the age of the animal and in a similar pattern.

METHODS

Rats of the Sprague-Dawley strain were fed a standard laboratory diet (D & G, The Price-Wilhoite Co.). The approximate time of conception was determined by vaginal smear the morning after an overnight period of mating. Fetal rats were obtained by hysterotomy under ether anesthesia. Older animals were killed by decapitation without anesthesia.

All animals 20 days of age or older were virgin females. In assays involving fetal and newborn animals the livers of one to three litters were used for each experiment. Freshly excised livers were

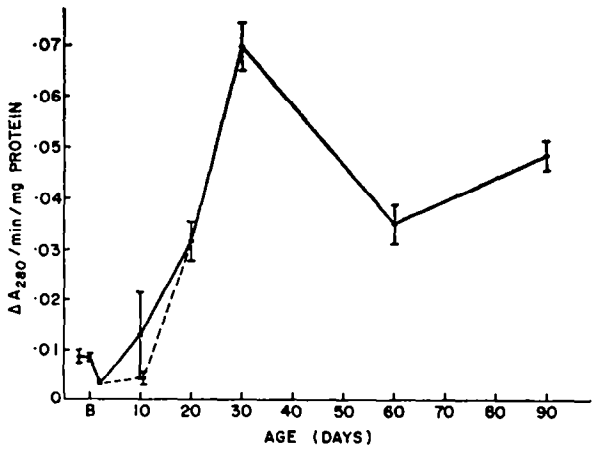

Fig. 1. The variation of glycine acyltransferase activity of rat liver with age. "B" indicates time of birth. Means ± S.E. are given. The broken line indicates value calculated when one markedly deviant result was omitted from the data on 10-day old animals.