by MEA, leading to decreased barbiturate degradation during the critical 0-15-minute period. It is known that high concentrations of MEA inhibit hexobarbital metabolism by rat liver enzymes(7,8).

The fact remains that a more direct, slowly developing, depression of the CNS, in correct time relationship with barbiturate depression, might have accounted for the additive effect. This explanation would be inconsistent with the lack of additive toxicity when pentobarbital was injected prior to MEA.

Summary. 1. MEA synergizes with pentylenetetrazole to produce death in mice when it is injected simultaneously with it, or at 15, 30, 60, or 120 minutes preceding it. Thus, MEA has a stimulant action which comes on rapidly after intraperitoneal injection and endures for at least 2 hours. 2. A central depressant, pentobarbital, does not interact lethally with MEA when injected prior to MEA. When MEA was injected simultaneously with or 15, 30, or 60 minutes prior to pentobarbital, it increased pentobarbital lethality. These results are most logically explained by assuming competitive interference with barbiturate metabolizing enzymes by the agents.

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Received July 21, 1966. P.S.E.B.M., 1966, v123.

Renal Changes in Dietary Hepatic Injury in Rats.* (31598)

PAUL GYÖRGY, W. E. EHRICH, AND B. W. LANGER, JR.†

Departments of Pediatrics and Pathology, Philadelphia General Hospital, Philadelphia, Pennsylvania

In previous studies(1) renal changes have been observed in rats fed cirrhosis producing rations. These changes were concentrated mainly in the convoluted tubules of the cortex, showing in the acute stage an alteration which was interpreted as "acute necrotizing nephrosis." There was in many instances distinct correlation between changes of the liver and of the kidneys, especially in the experiments using methionine. Supplement of methionine prevented both the hepatic and renal changes. This parallelism extended to some extent also to observations with basal ration in these experiments(1) which contained vitamin-free casein in low proportion (8-10%) as sole source of protein. Vitamins A and D were given mostly as cod liver oil mixed with the diet (2%) or 3 drops of percomorph oil per week. Thiamine HCl 20 μg, riboflavin 20 μg, pyridoxine-HCl 20 μg, Capantothenate 100 μg, and menadione 20 μg all dissolved in 1 ml were given 3 times weekly (twice 2 ml and once 3 ml) in small dishes separately from the diet. The fat content was rather high (22-40%): lard or Crisco (hydrogenated cottonseed oil). In general more severe hepatic and renal changes were found when lard and cod liver oil were given compared with Crisco. This was related to the vitamin E content of Crisco. Supplements of tocopherol have also reduced the incidence and severity of the renal changes. However, this protective effect of Crisco or of α-tocopherol was never complete and in many experiments (even 30 μg of α-tocopherol in daily supplement) statistically not significant.

* Supported in part by Grants A-5921 and T1-HD-17 of Nat. Inst. Health.

† Present address: SEATO Medical Research Laboratories, Bangkok, Thailand.

In more recent studies, instead of the original low-casein diet a ration(2) was used with 6% vitamin-free casein, 30% methanol extracted peanut meal with Crisco or lard and cod liver oil as fats, sucrose and Salt Mixture USP XIV. The same vitamin supplement was given separately as in the previous experiments. Renal changes were almost completely absent on the diet with Crisco as fat, and were very severe with lard and cod liver oil. Addition of 3 mg of α-tocopherol acetate (3 times weekly), given separately, to the diet with lard and cod liver oil has reduced significantly the renal(2), but only to a smaller degree the hepatic changes.

The question arose whether 1) these renal changes belong in the category of rapid autolysis in the lining of the renal tubule as found in rats with vit. E deficiency(3-8) and 2) whether these renal changes would respond to lipotropic factors (choline, methionine, vit. B_{12}) which prevent hepatic injury and have (at least methionine) in the past exerted beneficial effect also on renal changes(1).

Experimental methods. Male rats of the Sprague-Dawley strain, 10 in each group, were used. The initial weight was, in Exp. I 92 g, Exp. II 96 g, Exp. III 91 g, Exp. IV 107 g, Exp. V 107 g, always in each group. The basal ration EPM V consisted of methanol extracted peanut meal 25 (containing 55% protein), vitamin-free casein 6, sucrose 45, Salt Mixture USP XIV 4, lard 18, cod liver oil 2, niacin 10 mg. The B-vitamins (only thiamine HCl, riboflavin, pyridoxine HCl, Ca-pantothenate) were given separately, in doses as given in the Introduction. EPM VI contained only methanol extracted peanut meal as source of protein 44 (containing 55% protein), sucrose 31, lard 18, Salt Mixture USP XIV 4, fat-soluble vitamins (A, D, E) in soyabean oil 2, vitamin mixture and zinc acetate 675 mg/kg. Ten mg α-tocopheryl acetate (Roche), 1250 IU vit. A and 180 IU vit. D were all dissolved in 2 ml pure soyabean oil and added in this amount per 100 g of diet. The mixture of water-soluble vitamins consisted of thiamine-HCl 0.5 mg, riboflavin 0.5 mg, pyridoxine-HCl 0.5 mg, Ca-pantothenate 5.0 mg, niacin 8.0 mg, inositol 100.0 mg, p-aminobenzoic acid 100.0 mg, folic acid 0.025 mg, biotin 0.02 mg, ascorbic acid 10.0 mg, menadione 0.5 mg and sucrose up to 1 g. One gram of this mixture was added per 100 g of the ration.

At the end of the experimental period the rats were killed with ether in batches of 20-30 and autopsied. The kidneys were cut longitudinally in 2 pieces, the livers opened with several incisions and put in neutral formalin solution. This procedure could have required about ½-1 hour. The penetration of formalin throughout the organ might have also been delayed. Thus, the possibility of rapid autolysis, as described by Emmel(6,7) was not excluded.

Slices of liver and kidneys were embedded in paraffin and sections were stained with hematoxylin and eosin, carbol fuchsin, oil red and Beyer's trichrome stain. Sections of some kidneys were stained also with phosphatungstic acid and Ritter and Oleson's mucopolysaccharide stain. In most cases also frozen sections were made and stained with oil red.

Results. The microscopic appearance of the renal changes is illustrated in Fig. 1. They closely resembled the autolytic changes described and illustrated by Moore and associates(3-5,8) and by Emmel(6,7). There was no coagulation necrosis. There was no vital response to the dissolution of the tubular epithelium. There was no infiltration with polymorphonuclear leukocytes. There was no extravasation of erythrocytes.

Pertinent findings in liver and kidneys in 5 experiments are summarized in Table I. They clearly show that in rats fed the basal ration EPM V, not containing vit. E, renal autolysis was a regular occurrence. However, addition of crude liver extract, choline, vit. B_{12} (0.1 μg daily) suppressed not only the hepatic but also the renal changes, without additional vit. E. Methionine given separately was without effect either on the hepatic or the renal changes (Exp. II). Purified liver extract with a vit. B_{12} content of 0.07 μg prevented the renal but not the hepatic changes. No significant renal alteration was noticed in any of the experimental groups in the presence of α-tocopherol (Exp. V).

Comments. From the present and previous

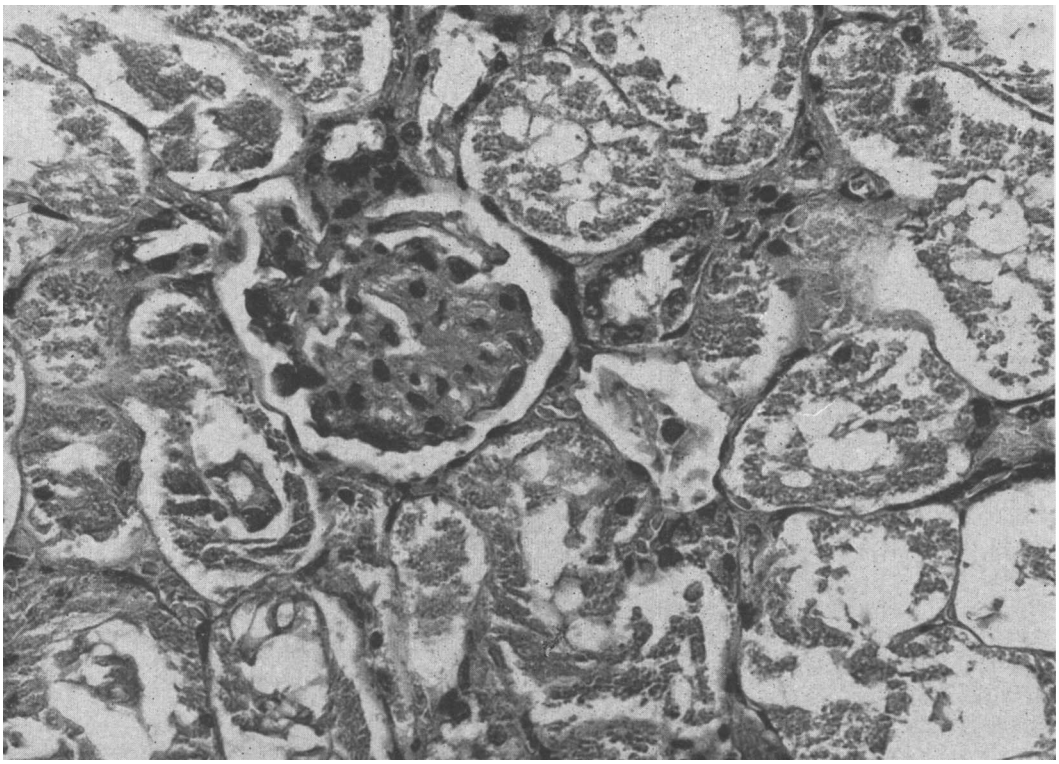

FIG. 1. Renal changes in dietary hepatic injury in rats.

studies(1,2) the conclusion emerges that renal changes, involving chiefly the cortical tubules, best described as autolysis, have a dual etiology: a) lack of vit. E and b) lack of lipotropic factors in the diet. The rations used in this series of observations differed from the basal diet used in the first experiments on the productions of dietary hepatic injury (1) in one respect: They were high in protein. However, the protein was either of poor biological quality (methanol extracted peanut meal) or a mixture of the same protein with the admixture of a small amount of casein (25% peanut meal and 6% casein). These mixtures supported much better growth than the original low casein diet (8-10%) and enhanced the development of hepatic and renal changes. In contrast, the ration used by the authors(3-8) for the production of early tubular autolysis in the kidneys as sign of vit. E deficiency were high in good quality protein (casein 20% or more), with the B-vitamins represented only by yeast in the diet, thus without vit. B_{12}. For the better production of renal changes, all authors stressed the necessity of polyunsaturated fatty acids in the diet (linoleic acid or the highly unsaturated fatty acids as present in cod liver oil). There is also a special relation between sulfur amino acids, vit. E and free radical peroxidation(12). In this connection, however, the difference between cystine and methionine should be mentioned, *i.e.*, cystine(1,13) enhances hepatic and renal changes, whereas methionine will prevent them(4), provided it is mixed with the rations EPMV and EPM VI. On a ration low in casein, methionine will prevent hepatic and renal changes even when added separately from the diet(1).

No explanation is available for the dual etiology of renal tubular autolysis in rats fed cirrhosis producing rations.

Summary. The renal changes observed in rats fed a cirrhosis producing diet manifest themselves in autolysis of tubular cells. They appear to be identical with similar changes reported in vit. E deficiency as a rapid post mortem occurrence. Supplements of vit. E

TABLE I

Exp	Group	Basal diet	Supplement	Liver fibrosis 0	Liver fibrosis +	Renal tubular changes 0	Renal tubular changes +
I	1	EPM V	None	1	9	0	10
	2	"	1 ml purified liver extract* inj daily	1	9	9	1
	3	"	20 mg crude beef liver† oral daily	9	1	10	0
II	1	EPM V	None	0	10	0	10
	2	"	1 μg B_{12} daily, inj	10	0	10	0
	3	"	20 mg crude beef liver oral daily	6	4	10	0
	4	"	25 mg/day choline	10	0	10	0
	5	"	25 mg/day methionine	1	9	1	9
III	1	EPM V	None	0	9	0	9
	2	"	1 μg B_{12} daily, inj	10	0	10	0
	3	"	25 mg/day choline	10	0	10	0
IV	1	EPM V	None	0	10	0	10
	2	"	.03 μg B_{12} daily, inj	6	4	9	1
	3	"	.1 " " " "	10	0	10	0
V	1	EPM VI	None	0	9	9	0
	2	"	Choline in diet 0.24%	10	0	10	0
	3	"	Choline separately	10	0	10	0
	4	"	Methionine in diet 0.3%	10	0	10	0
	5	"	Methionine separately	1	9	10	0
	6	"	B_{12} in diet 5 μg %	9	1	10	0
	7	"	B_{12} separately	1	9‡	10	0

* "Ripason" of Robapharm, Basle, Switzerland. Vit. B_{12} content 0.002 μg. Dry residue 28.5 mg/ml.

† Wilson's crude liver extract. Vit. B_{12} content 0.14 μg/20 mg.

‡ Mostly mild fibrosis.

Duration in all experiments 140 days. For further details see *Experimental methods.*

will prevent this tendency to rapid autolysis. Lipotropic factors (choline, methionine, and vit. B_{12}) are equally active, (methionine only when mixed with the diet) even in the absence of vit. E.

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