Occurrence of Hemorrhagic Centrolobular Liver Necrosis in Protein Deficient Rats.* (25565)

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During study of the pancreas of rats on synthetic but otherwise complete protein-deficient diets, changes in several organs were observed, particularly occurrence of centrolobular necrosis in livers of some animals. The important relationship of dietary liver cell necrosis for development of permanent liver damage has been recently emphasized by Bras, Goldblatt and György(1). For this reason, and also because central hemorrhagic necrosis is quite commonly found in livers of animals given dl-ethionine, a presumed methionine antagonist, a more detailed investigation in the incidence of this lesion in complete and partially protein-deficient rats was made. The influence of added cystine and methionine was also studied.

Material and method. Young male rats of Wistar strain weighing 120-170 g were divided into 9 groups. They were given a basic protein-free diet which contained corn starch 70%, vegetable oil 10%, cellulose 15%, cod liver oil 1% and salt mixture U.S.P. No. XIV. Vit. supplements/kg of food were added as follows: Vit. A concentrate 20.000 U, Viosterol 11.000 U, Inositol 110 mg, alpha-tocopherol 110 mg, choline chloride 1.65 g, Menadione 49 mg, para-amino benzoic acid 110 mg, niacin 100 mg, riboflavin 22 mg, pyridoxine 22 mg, thiamine 22 mg, calcium pantothenate 66 mg, biotin 0.44 mg, folic acid 2 mg, Vit. B_{12} 0.03 mg. Group 1 received the basic protein-free diet while Groups 2 to 8 received various food supplements in addition (Table I). In Group 9, 60% of vegetable fat (Crisco) was substituted for equivalent amount of starch in diet which contained 7.5% casein. Rats were weighed and sacrificed after 43 to 55 days. Slices of liver, kidney and pancreas were fixed in Bouin's solution as well as in 10% formalin and stained with hematoxylin-eosin. Iron and fat stains were done when indicated. Necrobiotic changes which occurred in many livers were graded as follows: ± when only isolated cells in close vicinity to the central vein were involved, and +1 when necrosis of from 1 to 2 rows of cells occurred. More extensive lesions were classified as +2. The severest degree of central necrosis involved to one-half of lobe was considered +3.

Results (Table I) - Group 1. Typical microscopic changes of protein depletion as described by various investigators(2,3,4) were seen in the liver of 60% of the rats. Liver cells appeared large and swollen. The cytoplasm appeared vacuolated and rarified due to considerable decrease of basophilic granules, while cell membranes stained prominently. The nuclei lost their vesicular appearance and became somewhat hyperchromatic. The sinusoids were narrow and often completely compressed. Fatty changes were also frequently observed. In addition, a centrolobular necrosis graded +1 to +3 was seen in about ½ of all animals. This necrosis was accompanied by fresh hemorrhages in central areas. The extension of such hemorrhages was in most instances proportional to necrotic damage. However, some livers presented extensive hemorrhages with minimal necrosis and vice versa. Infiltration by lymphocytes and large mononuclear cells was sometimes observed in necrotic areas. Occasionally iron containing histiocytes was seen, obviously indicating older hemorrhages.

Group 2. Liver cells showed a similar degree of rarifaction of the cytoplasm, as observed in Group 1. There was no significant difference in regard to incidence and severity of the centrolobular necrosis, since +1 to +3 lesions were encountered in 55% of the animals.

Group 3. Rarifaction of cytoplasm was seen in 2 out of 8 animals. Central necrosis judged +1 to +2 was present in 2 animals. No +3 lesion was observed in this group. Mild fatty changes were present in 1 animal as minute fatty droplets without zonal distribution.

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TABLE I. Summary of Findings in Groups of Rats on a Protein-Free Diet with and without Various Supplements.

Group	Diet	No. of rats	Days on diet	Initial wt, g	Wt changes, g	No. of rats showing necrosis				% of rats showing	
						0	±	1+	2+	3+	0 & ± lesions	1+ to 3+ lesions
1	Basic protein-free	34	43–55	120–170	All lost 45–90	10	9	5	9	1	56	44
2	*Idem* + 2% casein	22	"	120–160	*Idem* 35–85	6	4	7	5	0	45	55
3	" + 4% "	8	49–55	130–160	" 30–50	6	0	1	1	0	75	25
4	" + 7.5% "	13	45–55	140–170	12 lost 10–30 1 no change	6	6	1	0	0	92	8
5	" + 1% methionine	14	46–55	"	All lost 40–90	4	2	3	4	1	43	57
6	" + 2% casein + 1% methionine	14	"	150–160	*Idem* 50–70	1	2	3	5	3	21	79
7	" + .5% cystine	14	46	160–170	" 50–80	1	0	4	5	4	7	93
8	" + 2% casein + .5% cystine	7	45–55	140–160	" 20–60	0	1	3	3	0	14	86
9	7.5% casein diet 60% fat	14	"	150–160	5 lost 10–20 6 unchanged 3 gained 40	4	2	5	3	0	42	58

Group 4. Central necrosis was almost completely absent since only 1 out of 13 animals presented a +1 lesion. Typical cytologic changes of protein depletion were seen in only 1 case. Fatty changes were minimal in 2 rats.

Groups 5 and 6. These 2 groups can be discussed together as the effects on these diets were similar. Typical protein depletion changes in liver cells were observed in most animals. Fifty-seven and 79% respectively of livers showed +1 to +3 central necrosis. Fatty changes ranged from minute fatty droplets in occasional liver cells with or without zonal arrangement to severe fatty infiltration as large droplets in many liver cells, mainly affecting cells in periportal areas.

Groups 7 and 8. No significant difference was seen in these 2 groups. Typical changes of protein depletion of liver cells were seen in both groups in 30% of the animals. Centrolobular necrosis graded +1 to +3 was encountered in 93% of rats in Group 7 and 86% in Group 8, accompanied by extensive recent hemorrhages. Fifty % of animals in both groups showed also histiocytes containing hemosiderin pigment in the centrolobular areas. Mild fatty changes were seen in few animals, located chiefly in periportal and mid-zonal areas.

Group 9. Fifty-eight % of rats presented central necrosis graded +1 to +3 with extensive hemorrhages. Fatty changes of liver cells were severe and observed in all animals. These fatty changes were limited to periportal liver cell cords but extended in several instances into mid-zonal areas. In contrast, cytoplasmic vacuoles of liver cells due to protein depletion present in all instances were also observed in central areas.

Discussion. On complete protein deficient diet liver cells show rarifaction of cytoplasm due to disappearance of their basophilic granules, the so-called protein storage particles of Berg which are thought to be ribonucleic acid aggregates(4). In these livers increase in glycogen and lipids has been reported(4,5). In our material fatty changes varied from a very slight to a fairly marked degree. Rarifaction of cytoplasm and fatty changes were

not essentially influenced by addition of 2% casein while a supplement of 4% casein led to distinct improvement in the microscopic appearance. When the diet contained 7.5% casein, liver cells were almost normal, provided fat content was not unduly raised. If 60% of carbohydrates were replaced by equal amount of fat in the diet, rarifaction of cytoplasm, associated with severe fatty changes, was again noted(6).

Centrolobular necrosis was present in rats on complete protein-free and 2% casein diet. There was a protective influence of 4, and more marked of 7.5% casein. Addition of methionine or cystine did not prevent the lesions. Cystine even aggravated severity of centrolobular necrosis. This is probably due to a direct necrotizing effect of cystine(7,8).

Few investigators have studied livers of rats on severely protein-deficient diets. Central necrosis was not found by Kosterlitz in rats on a protein-free diet to 28 days(4), or by Jaffe *et al.*(9), who kept their rats to 111 days on a diet containing only 2% protein. Centrolobular necrosis was reported in some experiments containing 4 to 6% casein(10,11). However, these diets were deficient in Vit. E. György and Goldblatt(12,13), fed as much as 18% casein, and observed a variety of hepatic lesions including central and mid-zonal necrosis. Addition of yeast or Peter's eluate, however, completely prevented occurrence of these lesions. Massive necrosis of liver cells seen when highly purified casein or certain yeast proteins are fed, is obviously of different etiology, since it can be prevented by either of 3 well defined factors, including Vit. E(14). The very occasional occurrence of focal liver cell necrosis in a diet containing 9% casein, all essential vitamins and alpha-tocopherol reported by Herrera *et al.*(15) is in agreement with our findings of occasional necrosis in rats consuming sub-optimal amounts of protein. Centrolobular necrosis in combination with hemorrhages in a substantial percentage of our animals on protein deficient diets is of considerable interest in view of the microscopically similar centrolobular necrosis following administration of dl-ethionine. It is generally assumed that dl-ethionine acts as a methionine antagonist, since addition of equimolecular amounts of methionine to the diet completely prevents ethionine lesions. Such a favorable effect of methionine was not seen in protein deficient animals. Other hepatic lesions which regularly occur in animals given ethionine, *i.e.*, characteristic proliferation of "ductular cells" and of regenerative changes were not seen in our animals(16,17). This again indicates that the ethionine lesion is probably of a complex nature and not only due to a conditioned methionine deficiency(18). Rats force-fed diets deficient in certain amino acids developed fatty infiltration, predominantly periportal in distribution, but not necrotic changes(19,20).

Central necrosis has been described in totally starved mice(21). It is, however, unlikely that the changes in our rats were due to starvation, since they were also seen in rats given 7.5% casein and 60% fat. The majority of animals in this group lost no weight and some even gained weight.

We previously described atrophic lesions of the pancreas on protein deficient diets and found that addition of 2% casein was without influence, while 4% casein or 1% methionine had a marked beneficial effect(22). In the present investigation, we again found considerable improvement on the 4% casein diet. However, we were unable to reproduce our previous finding of a beneficial effect of a methionine supplement.

Summary. On a synthetic protein-free diet containing all other essential nutrients with or without a 2% casein supplement, the livers of young male Wistar strain rats show centrolobular necrosis, often associated with hemorrhages in about 50% of animals. Incidence of these lesions decreases when casein content of diet is increased. Addition of 1% methionine is of no influence, while 0.5% cystine often aggravates the severity and incidence of centrolobular necrosis. The findings are discussed with reference to hemorrhagic centrolobular necrosis which occurs frequently after administration of ethionine, a supposed methionine antagonist.

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Effect of Krebs-Ringer Medium on Muscle Contraction *In situ* in Adrenalectomized Albino Rats. (25566)

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Although it is well established that adrenal cortical insufficiency is accompanied by reduced ability for sustained muscle performance *in vivo,* underlying mechanisms are not clearly understood. Tensile(1,2), electrical (2), or biochemical(3) properties of resting muscle are apparently not sufficiently altered in adrenal insufficiency to be implicated in any major way as direct targets of corticoid action. Nevertheless, corticoids normally may act directly on contractile processes to a limited extent(4). Although weight of evidence (5,6,7,8,9) suggests that impaired muscle performance *in vivo* in adrenal insufficiency is related to well known accompanying circulatory and cardiovascular disturbances(10,11), it is not certain whether these contribute directly by acting on contractile processes or indirectly by interfering with circulatory adjustments normally occurring under physiological demands for sustained muscular activity. Whatever the underlying physiological mechanism, immediate cause of impaired muscle function may be consequential to inadequate transport of metabolic substances vital to sustained muscle activity or to inadequate removal of toxic metabolites. The immediate importance of circulatory factors is suggested by the work of Ramey, Goldstein, and Levine (5) who have shown that differences in work performance *in vivo* of intact and adrenalectomized rats fatigued by swimming are not reflected in diaphragm and oblique abdominal muscles isolated to nutrient medium. However, in these studies differences in muscle work performance prior to isolation were assumed and not demonstrated. In the present paper we have obtained quantitative data from *in situ* experiments designed to demonstrate more definitively the effect of nutrient medium on muscle contractile capacity in adrenalectomized rats.

Methods. Male Rockland-Sherman rats weighing approximately 200 g were bilaterally adrenalectomized or sham operated under

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