(debittered, dried brewers yeast), which do not induce liver degeneration, exert a strong protective influence when added to diets causing the disease. It has been demonstrated by fractionation experiments that this effect is not due to vit. E or cystine, but due to a third unidentified factor, designated factor 3, which has been concentrated from yeast K. (2) The presence of factor 3 explains why dietary necrotic liver degeneration cannot be produced with American yeasts G and K. Factor 3 is water soluble, stable against acid hydrolysis, and is not identical with any one of the presently well-known vitamins.

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Free Amino-Acids in Rat Brain During Insulin Shock. (19241)

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It has been reported(1) that after insulin administration, the rat's brain content of free glutamic acid lessens, free amino acid nitrogen is slightly diminished, but no changes occur either in glutamine or ammonia. The explanation suggested was that during the severe conditions accompanying hypoglycemia, glutamic acid is oxidized, and that ammonia is in some way metabolized or liberated from the brain, without any change taking place in other free amino acids. Nevertheless, the authors considered it necessary to submit this last assertion to the test of experiment, particularly in connection with amino acids closely related to glutamic acid in tissue metabolism, such as aspartic and γ-aminobutyric acids, the latter recently found in brain as a decarboxylation product of glutamic acid(2,3).

Experimental. Nine groups, each with 6 to 8 young male albino rats each weighing 100 to 150 g were used. Rats of groups 1-7 received no food (water *ad libitum*) during 24 hours, and then, to half the rats, subgroup B, 350 IU/k of Lilly's insulin were injected intraperitoneally, the other subgroup A, being kept as controls. Starvation conditions were further maintained, in the subsequent period, during which insulin shock developed in subgroups B, and some of their members died. Rats of groups 8 and 9 were not starved before their subgroups B were injected with 60 IU/k insulin, thus maintaining them under conditions similar to those in Dawson's ex-

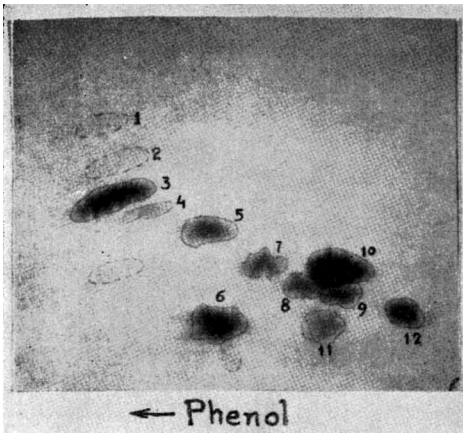

FIG. 1

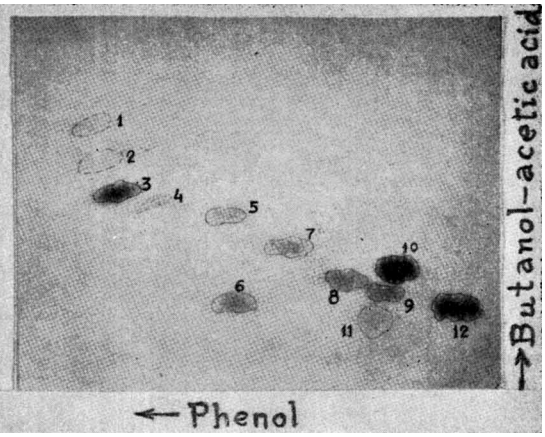

FIG. 2

FIG. 1 and 2. Two chromatograms from rat brain extracts, normal (1) and during insulin shock (2). Spots numbered: 1, leucine and isoleucine; 2, valine; 3, γ-amino-butyric acid; 5, alanine; 6, glutamine; 7, threonine; 8, glycine; 9, serine; 10, glutamic acid; 12, aspartic acid. 4 and 11, unidentified.

periments(1). At the end of the 4 hour period for groups 1-7, and only 2 hours for groups 8 and 9, rats still alive were killed by cervical vertebral dislocation. The brains from members of each subgroup were immediately dissected, pooled, and homogenized in a Waring Blendor with 80% ethyl alcohol. The resulting suspensions, after treatment by Awapara's method(4), furnished protein and lipid-free extracts, some of which were desalted by electrodialysis in an apparatus assembled on the principles followed by Consden *et al.*(5). After a 10-fold concentration, by boiling, extracts with 200-400 μg of amino acids/0.01-0.02 ml were finally used for running threefold series of chromatograms on Whatman No. 1 filter paper, by the bi-dimensional descending technic of Consden *et al.* (6), in the phenol-80% butanol-acetic acid-water (4:1:1) system. Filter paper sheets were dried in a current of heated air, and sprayed with a dilute solution of ninhydrin in butanol. *Rf* values for the spots of glutamic, aspartic and γ-amino-butyric acids, and glutamine, were checked by running chromatograms of standard solutions of amino acids, as well as by adding them to brain extracts. Concentrations of each amino acid and glutamine in the spots were measured by the colorimetric method of Naftalin(7) in a Coleman Junior spectrophotometer, at 570 mμ, glutamic acid being used as a standard for glutamine. Total amino acid nitrogen was measured in duplicate samples of some of the extracts, by Sahyun's colorimetric method(8). All steps were carried out simultaneously for brains from paired A and B subgroups.

Results and discussion. Inspection of Fig. 1 and 2, and of Table I, shows that brain extracts of insulin injected rats have, as compared with those from untreated animals, lesser amounts of free glutamic acid, γ-amino-butyric acid and glutamine, and conversely, a greatly increased content of free aspartic acid and practically no change in the free amino acid nitrogen level.

On the whole, our results are in agreement with those of Dawson regarding glutamic acid and amino acid nitrogen, but introduce the new finding of the increased free aspartic acid, which requires an equally new explanation.

Contraposition of such noticeable increase in free aspartic acid with the decrease in glutamic acid level, associated with the lack of important changes in total amino acid nitrogen, suggests that under the experimental conditions, the increase in aspartic acid may be due to activation of a transamination reaction between glutamic and oxalo-acetic acids. α-ketoglutaric acid, another product of the same reaction, probably increases under the emergency conditions accompanying hypoglycemia and enters the tricarboxylic acid cycle. The great importance of such a transamina-

TABLE I. Free Amino Acids, Total Amino Acid Nitrogen and Glutamine in Brain of Rats, Normal and During Insulin Shock.

Groups	Hr of starving	IU/k	mg/100 g brain tissue: Total amino acid, N A	Total amino acid, N B	Glutamic acid A	Glutamic acid B	Aspartic acid A	Aspartic acid B	γ-amino-butyric acid A	γ-amino-butyric acid B	Glutamine A	Glutamine B
1	24	350	—	—	168.4	127.9	37	110	29.1	20.6	43.7	42.2
2	24	350	—	—	166.6	124.1	52.8	161.7	43.4	32.7	40.5	13.5
3	48	350	39.6	33.9	173.1	127	40	146.3	30.9	17.3	31.6	11.7
4	24	350	—	—	166	77	42.7	84.4	44.8	22.4	33.5	7
5	24	350	37.2	29.9	194.7	136.4	44.8	66	30.1	22.5	41.4	9
6	24	350	45	44.8	146.3	88.9	34.3	138.6	31.7	19.5	34.5	6.5
7	24	350	33	30.5	188	150.7	42.7	55.9	24.7	16.2	—	—
8	0	60	31	30.5	160.9	147.4	41.6	58	38.9	30.6	32.4	17
9	0	60	30.8	30.1	189.2	166.1	40.1	81.2	41.6	25	—	—

A, control rats; B, rats under insulin shock.

tion system in normal brain tissue, has been pointed out by Cohen and Hekhuis(9).

Increased activity in transamination between glutamic and α-ketoglutaric acids explains, furthermore, why the ammonia level remains practically unaffected during insulin hypoglycemia, and makes the lowering of γ-amino-butyric acid and glutamine levels, referable to diminution in available free glutamic acid.

Summary. Brain extracts of rats subjected to insulin shock have, as compared with those from normal animals, smaller amounts of free glutamic acid, γ-amino butyric acid and glutamine, and a greatly increased quantity of free aspartic acid.

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Effect of Vitamin E and CCL_4 on Fat, Respiration and Choline Oxidase of Rat Livers.* (19242)

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A previous report(1) indicated lower liver fat in young rats deficient in vitamin E as compared with controls receiving this factor. On a diet low in protein and deficient in vitamin E, young rats are known(2,3) to be highly sensitive to acute CCl_4 toxicity; this condition is corrected by supplements of alpha tocopherol. CCl_4 is known to produce an immediate fatty infiltration of the liver, and Ennor(4) has shown an increased rate of respiration of liver slices from guinea pigs poisoned with CCl_4. Therefore, the influence of vitamin E on the response of liver fat and the rate of liver slice respiration after acute CCl_4 intoxication has been investigated in rats.

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