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Serum and urine amino acid pattern under the effect of carbon disulfide intoxication

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With 4 tables

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Inhalation of carbon disulfide either through the respiratory tract or the skin (4, 6) is deposited in the different tissues including the liver, brain, lung, heart, and kidney (10, 3). Hypertrophy of hepatic cells, followed by degeneration changes, have been reported by *Mezzasalama* and *Cerbone* (13). In experimental animals, changes varied from diffuse fatty infiltration to the picture of chronic aggressive hepatitis (2, 8).

The histopathological changes in the kidney showed increased incidence of chronic interstitial nephritis when compared with control rats. This varied from a mild cellular infiltration to marked diffuse medullary fibrosis (5, 8). Such pathological changes have shown to depend on the degree of exposure (12). Liver and kidney dysfunction would affect amino acid metabolism in a normal rat. Furthermore, carbon disulphide in the body is known to react with amino acids and peptides to form thio derivatives and such a process would affect the rate of utilization of such amino acids in the body (21, 17). However, as far as available literature could tell, no information about how far the body can make use of amino acids under the effect of carbon-disulfide intoxication.

The aim of this work is to throw some light on the amino-acid pattern in serum and urine under the effect of carbon-disulfide intoxication in rats as compared to controls. Besides, the effect of dose stoppage upon reversibility of such pathogenic alteration in amino-acid pattern is also investigated.

Materials and methods

The material of this study comprised 60 rats of both sexes weighing 120–150 g. Rats were categorised into six groups each of ten. They were injected intramuscularly with a dose of 0.05 ml carbon disulphide in 0.2 ml olive oil daily over a period of 50 days, during experimentation, rats were fed laboratory diet ad libitum (15). Every 10 days, rats of one group were killed by decapitation and blood was collected.

Twenty rats of similar weight were included, fed on the same diet, injected with 0.2 ml olive oil alone to serve as controls. Four of them were killed with each of the injected groups. Carbon-disulfide injection was stopped for group six after the 50th day of the experiment to test the extent of regression of the developed biochemical derangements.

Plasma- and urine-amino acids were determined by the two-dimensional paper chromatographic technique as described by *Levy* and *Chung* (11).

Results

In carbon-disulfide-intoxicated rats variable hypoaminoacidemia affecting both essential and non-essential amino ecids could be demonstrated. Table 2 shows the percentage decrease in individual amino acids in carbon-disulfide-injected rats relative to controls. Table 3 shows the levels of total and individual amino acids in urine of controls and the intoxicated rats.

Under the effect of carbon-disulfide intoxication, hypoaminoacidemia was accompanied by hyperaminoaciduria in all groups of rats, and table 4 summarises the percentage increase in amino acids in urine of intoxicated rats relative to controls.

Data obtained for group 6 reveal that most plasma and urine amino acids started to return to the normal levels after 20 days following 50 carbon-disulfide injections.

Discussion

Plasma-amino acids are mainly derived either from exogenous dietary sources or from endogenous sources and the pattern of plasma-amino acids can be thus affected by different mechanisms. The extent of variations of plasma-amino acids depends to a great extent on the degree of participation of these different factors.

In our study, individual free amino acids either essential or non-essential showed a decrease especially in rats of group 5. However, the different amino acids were affected to variable extent.

Phenylalanine was the most affected amino acid, followed by glutamine, aspartic acid, lysine, alanine, asparagine, tyrosine, valine, threonine, cysteine, glycine, leucine, glutamic acid, serine, and methionine. It is clear from such pattern that the degree of affection on amino acids does not follow a regular system. Some of the essential amino acids as phenylalanine, lysine were highly affected while other essential amino acids as methionine, valine, leucine are not markedly affected. On the other hand, the non-essential amino acids aspartic acid, asparagine, tyrosine were much more changed than other non-essential amino acids as cysteine, serine, glycine, and glutamine.

The decrease in plasma-amino acids may be due to shortage of amino-acid supply from dietary sources as a result of decreased food consumption, as a result of loss of appetite (9). Pathological changes in the liver reported by *Eisa* et al. (8) may support this phenomenon. The interaction of carbon-disulfide with amino groups of amino acids and protein to form thiocarbamate which cyclise to thiosolidone (18, 16, 5) may be a contributing factor. The chelating effect of dithiocarbamates and thiosolidone with metals (7) may cause amino acids to be non-available to the organism and may also facilitate its secretion from the kidney.

The essential amino acids in toto are not markedly affected as the non-essential amino acids. This may indicate a blockage in protein synthesis which may be due to enzymatic deactivation (14, 20). In this respect, it is

Table 1. Free amino-acid concentrations (mg/100 ml plasma) of controls and CS_2-intoxicated rats (mean ±SE and >P).

Group	Asp	Glut	Ser	Gly	Ala	Thr	Glm	Val	Meth	Leu	Tyr	Cyst	Aspg	Hist	PhAl
Con-	0.38	1.23	1.60	1.54	1.50	1.34	0.73	0.99	1.14	1.63	1.87	0.57	1.35	0.59	0.95
trols	±0.05	±0.08	±0.09	±0.09	±0.11	±0.08	±0.02	±0.11	±0.11	±0.15	±0.06	±0.03	±0.13	±0.08	±0.08
	0.36	1.27	1.49	1.42	1.35	1.27	0.63	0.93	1.12	1.50	1.62	0.55	1.24	0.58	0.75
I	±0.07	±0.08	±0.09	±0.10	±0.11	±0.13	±0.07	±0.14	±0.18	±0.06	±0.13	±0.10	±0.11	±0.07	±0.09
	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
	0.30	1.18	1.44	1.38	1.35	1.21	0.56	0.86	1.10	1.43	1.56	0.51	1.12	0.45	0.60
II	±0.07	±0.08	±0.08	±0.05	±0.10	±0.14	±0.06	±0.08	±0.09	±0.06	±0.13	±0.06	±0.13	±0.04	±0.11
	0.15	0.15	0.10	0.10	0.10	0.15	0.05	0.10	0.15	0.10	0.10	0.15	0.10	0.10	0.10
	0.27	1.06	1.38	1.25	1.20	1.11	0.46	0.79	1.01	1.39	1.43	0.45	0.99	0.40	0.56
III	±0.06	±0.07	±0.05	±0.08	±0.11	±0.12	±0.06	±0.07	±0.12	±0.09	±0.10	±0.04	±0.11	±0.04	±0.09
	0.10	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.15	0.05	0.05	0.10	0.01	0.05	0.01
	0.24	0.98	1.34	1.18	0.98	0.99	0.42	0.72	0.94	1.31	1.35	0.43	0.94	0.37	0.44
IV	±0.06	±0.08	±0.09	±0.09	±0.12	±0.12	±0.06	±0.06	±0.09	±0.06	±0.10	±0.03	±0.10	±0.03	±0.09
	0.10	0.05	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.05	0.01	0.05	0.01
	0.21	0.86	1.25	1.21	0.95	0.93	0.40	0.65	0.91	1.26	1.25	0.39	0.82	0.31	0.41
V	±0.05	±0.06	±0.04	±0.13	±0.12	±0.11	±0.05	±0.06	±0.08	±0.06	±0.10	±0.05	±0.17	±0.02	±0.06
	0.05	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	0.21	0.99	1.32	1.25	1.04	0.95	0.39	0.66	0.98	1.33	1.29	0.40	0.98	0.36	0.43
VI	±0.05	±0.08	±0.05	±0.12	±0.13	±0.11	±0.06	±0.06	±0.08	±0.06	±0.08	±0.03	±0.15	±0.03	±0.07
	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.15	0.15	0.01	0.01	0.01	0.01	0.01

Table 2. The percentage decrease of free amino acids in plasma of CS_2-intoxicated rats relative to controls.

Group	Asp	Glut	Ser	Glyc	Glm	Ala	Thr	Val	Leuc	Meth	Lys	Tyr	Aspg	Cyst	PhAl
I	5.3	3.2	6.9	7.8	13.7	10.0	7.3	6.1	8.0	1.8	3.4	13.4	8.2	3.6	21.4
II	21.1	4.1	10.0	10.4	23.3	10.0	9.8	13.2	12.6	3.1	23.8	16.6	17.1	10.6	36.9
III	28.9	13.9	13.8	18.9	37.0	20.0	17.2	20.3	24.8	11.5	32.3	23.6	26.7	21.1	41.1
IV	36.9	20.4	16.3	23.4	43.5	35.7	26.2	27.3	19.7	17.6	37.3	27.9	30.4	24.6	53.7
V	44.8	30.1	21.9	21.5	45.3	46.7	30.6	30.9	22.7	20.2	47.5	33.2	39.3	31.6	56.9
VI	44.8	19.6	17.5	19.9	46.6	30.7	29.2	33.4	39.9	14.1	39.0	31.1	37.5	29.9	36.8

Table 3. Free amino-acid concentrations (mg/100 ml urine) of controls and CS_2-intoxicated rats (mean ± SE and <P).

Groups	Asp	Glut	Ser	Glyc	Ala	Glm	Val	Leuc	Tyr	Cyst	Hist	Arg
Con-	2.06	1.42	2.21	3.46	3.73	3.06	0.20	0.17	1.73	6.29	2.17	1.78
trols	±0.30	±0.22	±0.38	±0.41	±0.24	±0.33	±0.08	±0.05	±0.31	±0.28	±0.12	±0.32
	2.24	1.71	2.99	4.06	4.26	3.50	0.21	0.18	2.41	6.44	2.42	2.78
I	±0.18	±0.23	±0.54	±0.63	±0.45	±0.33	±0.07	±0.06	±0.53	±0.24	±0.26	±0.45
	0.15	0.15	0.10	0.15	0.15	0.05	0.15	0.15	0.15	0.15	0.15	0.10
	3.46	2.48	3.27	6.45	4.68	3.87	0.28	0.22	2.62	7.14	2.94	2.86
II	±0.26	±0.13	±0.21	±0.64	±0.51	±0.21	±0.08	±0.11	±0.32	±0.27	±0.48	±0.54
	0.05	0.10	0.05	0.10	0.10	0.05	0.15	0.15	0.15	0.15	0.15	0.05
	5.51	2.91	3.34	6.56	5.10	4.31	0.32	0.26	2.80	8.98	3.20	2.88
III	±0.21	±0.17	±0.54	±0.72	±0.34	±0.23	±0.10	±0.17	±0.56	±0.51	±0.50	±0.56
	0.01	0.01	0.01	0.01	0.05	0.01	0.15	0.05	0.15	0.01	0.15	0.05
	6.61	3.16	4.43	7.78	5.44	5.77	0.34	0.37	3.20	10.13	4.18	3.10
IV	±0.20	±0.22	±0.55	±0.63	±0.34	±0.27	±0.16	±0.10	±0.71	±0.89	±0.56	±0.46
	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.15	0.15	0.01	0.01	0.05
	7.85	4.15	5.97	10.81	6.36	6.15	0.29	0.39	3.52	14.13	4.42	3.72
V	±0.38	±0.25	±0.18	±0.69	±0.61	±0.48	±0.10	±0.24	±0.56	±0.97	±0.88	±0.56
	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.05	0.05	0.01	0.01	0.01
	6.31	3.48	4.20	7.03	5.53	4.20	0.26	0.28	2.69	11.40	3.50	2.69
VI	±0.36	±0.39	±0.68	±1.09	±0.89	±0.78	±0.18	±0.02	±0.58	±0.79	±0.81	±0.84
	0.01	0.01	0.05	0.01	0.05	0.10	0.15	0.15	0.05	0.01	0.05	0.05

Table 4. The percentage increase of free amino acids in urine of CS_2-intoxicated rats relative to controls.

Groups	Asp	Glut	Ser	Glyc	Ala	Glm	Val	Leuc	Tyr	Cyst	Hist	Arg
I	8.7	20.4	35.2	17.3	14.2	14.3	5.0	5.8	39.3	2.3	11.0	56.2
II	67.4	74.6	47.9	86.4	25.4	26.4	40.0	29.4	51.4	21.7	35.4	60.6
III	167.4	104.9	51.1	89.5	36.7	40.8	60.0	30.0	61.8	42.7	47.5	61.7
IV	220.8	122.5	101.4	124.8	45.8	88.5	70.1	52.9	84.9	61.0	92.6	74.1
V	281.1	192.2	170.1	212.4	70.5	100.9	40.5	117.6	103.4	124.6	103.6	107.9
VI	206.3	195.1	90.0	103.2	48.3	37.3	30.0	64.7	55.4	81.2	61.3	51.1

worth mentioning that the capacity of the liver with respect to protein synthesis have been reported to be hindered under the effect of carbon-disulfide intoxication (12).

The variable extent of decrease among the individual amino acids seems difficult to explain. However, it is suggested that this may be due to certain specific alteration in their metabolic pathways. Thus, the transformation of methionine to cysteine has been suggested to be minimized owing the availability of sulphide radical under CS_2 intoxication (19).

Carbon disulfide intoxication resulted a significant increase in the levels of all individual amino acids were detected in urine of intoxicated rats. A state of hyperaminoacidurea was observed in both essential and non-essential amino acids, particularly with those receiving multiple doses of carbon disulfide (tables 3 and 4).

It is of interest to mention that aminoacidurea affected all amino acids whether their levels were decreased in plasma or not, throw doubt on the possibility that aminoacidurea in these conditions is of the overflow type. On the contrary, such finding points to a renal laekage as an accessory factor in the occurrence of aminoacidurea. Our suggestion in this respect is that the aminoaciduria may be due to one or more factors. The first may be the disturbance in the processes of filtration in spite of the low levels of amino acids in plasma. In other words, the threshold of the kidney is decreased leading to increased filtration. The second may be due to decreased tubular reabsorption. Also increased loss of amino acids in urine as a result of either back decomposition of the thiocarbamate due to the release of tissue or as a part of the tissue degradation which occurs under the effect of carbon-disulfide intoxication (8).

Of value to add that the state of normalisation of amino acid levels on stoppage of CS_2 doses in group 6 is that the rats started to restore their appetite. Furthermore, improvement in amino-acid metabolism may be due to diminution in the concentration of chelating agent and recovery of the tissues.

Summary

The effect of carbon disulphide intoxication on amino acid pattern was studied. Five groups of rats were treated with carbon disulphide through intramuscular injection of 0.05 ml CS_2 in 0.2 ml olive oil/rat/day. A number of rats were sacrified after receiving 10, 20, 30, 40, and 50 injections.

As a result of intoxication, a state of hypoaminoacidemia and hyperaminoacidurea affected all the amino acids investigated, but to variable extent.

Of value to add that stoppage of carbon disulphide leads to improvement of the amino-acid pattern in group six, compared to the other groups.

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