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ABNORMAL METABOLITE IN ALCOHOLIC SUBJECTS

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ABSTRACT

An abnormal diol, 2,3-butanediol has been discovered in the serum of most but not all of 113 chronic alcoholic patients after ethanol ingestion.

Gas chromatographic analysis of deproteinized sera revealed a peak coinciding with ethanol in concentration ranging between 3 and 96 μM in 78 of 113 unselected patients admitted to an alcohol treatment center. Of the 78 patients with measurable blood ethanol on admission, 69 (88%) had a peak on gas chromatography coinciding with 2,3-butanediol in concentration ranging from 0.011-0.841 μM. No butanediol was found in the blood of the other 9 (12%) despite ethanol concentrations in the blood ranging from 5 to 85 μM. Similar analyses of sera from 54 controls, patients hospitalized with a variety of medical diagnoses other than alcoholism and including 8 with diabetes mellitus, disclosed no measurable blood ethanol and no 2,3-butanediol except that 2 control sera showed a very small peak coinciding with 2,3-butanediol near the lower limit of detection, 0.01 μM. Twenty-two (20%) of the 113 alcoholic patients had been independently diagnosed as manic depressive depressives. The blood of all but one contained 2,3-butanediol in concentrations ranging from 0.013 to 0.140 μM.

ABNORMAL METABOLITE IN ALCOHOLIC SUBJECTS

Higher alcohols including 2,3-butanediol have been identified in the blood of patients with hepatic coma and severe uremia, as well as in alcoholics suffering from lactic acidosis (Thölen, et al, 1961; Thölen, et al., 1962; Thölen, et al., 1962; Thölen, et al., 1962; Soling, et al., 1964 and Mammer, et al., 1978). Apart from these situations of severe metabolic derangements, 2,3-butanediol has been reported in this journal in the blood of physically healthy individuals suffering from manic-depressive psychosis (Dawson, et al., 1956; Dawson, et al., 1956).

The chemical measurements in these studies (Thölen, et al., 1961; Thölen, et al., 1962; Thölen, et al., 1962; Thölen, et al., 1962; Soling, et al., 1964; Mammer, et al., 1978; Dawson, et al., 1956; Dawson, et al., 1956) were performed by an old analytical technique. In a report published in 1977, gas chromatography indicated the presence of 1,3-butanediol in the blood of a patient who had displayed violent behavior following alcohol consumption (Altschule, et al, 1977). The measurements reported here were carried out by a new and highly sensitive method for measuring higher alconols in blood and were confirmed by mass spectrometry. The method is described in separate publications together with a discussion of the possible sources of the substances and their putative metabolic precursors (Felver, et al., 1980; Veech, et al., 1981).

METHODS

The subjects were 113 unselected alcoholic patients (27 females

and 86 males) ranging in age from 15 to 79 years admitted during a period of nearly 16 months between 11/29/78 to 3/12/80 to an alcohol detoxification center in Bethlehem, Pennsylvania. History and physical examinations were performed by the physician in charge of the unit who, together with a psychologist, made a clinical assessment of the mental and emotional state of the patient on admission and repeatedly throughout a 5 to 14 day residence of the unit. The clinical asssessments were performed entirely independently of the laboratory measurements.

Upon admission to the alcohol unit, specimens of blood were obtained for a variety of routine tests including gamma GT* and SGPT.** Separate samples of blood serum were drawn for the measurement of ethanol and the diols. The serum was separated, frozen and stored at minus 70°C. The specimens were then sent blinded to the Intramural Laboratory of Metabolism of the National Institute of Alcohol Abuse and Alcoholism. From 26 of the alcoholic subjects, subsequent blood specimens were obtained 18 hours later and analyzed in the same fashion. Fifty-four non-alcoholic healthy subjects (15 females and 39 males) between 27 and 81 years of age including 7 non-alcoholic diabetics (3 females and 4 males) between 46 and 72 years of age served as controls.

Ethanol concentration of whole serum was determined by a modification of the method of Baker (Baker, et al., 1969) using a 6-ft. porapak QS column operated at 125°C. The carrier gas was helium. At a flow rate of 30 ml/min ethanol eluted at 4.1 minutes free from interfering peaks.

* Gamma gutamyl transpeptidase
** Serum glutamic pyruvic transaminase

2,3-butanediol, 1,3-butanediol and 1,2-propanediol were measured in deproteinized sera using a 6-ft. column packed with Porapak PS coated with 3% Carbowax 20 M. Serum proteins were removed by precipitation with 0.5 M percholoric acid. The clear, protein-free supernatant was neutralized with KOH in order to remove perchlorate ion and to eliminate artifactual peaks often encountered in acidified extracts. 2,4-pentanediol (Aldrich Chemical Co., Milwaukee, WI) was arbitrarily chosen as the internal standard and was added to the serum extracts immediately prior to analysis. At 150°C, with a helium flow rate of 15 ml/min, 1,3-butanediol, and the internal standard were eluted at 5.2, 9.8 and 10.8 min, respectively.

The recovery of both isomers of butanediol and 1,2-propanediol from serum was 99% as determined by standard addition of the diols (Aldrich) to serum from control subjects before deproteinization. The identifications of 2,3-butanediol and 1,2-propanediol in serum samples from alcoholic patients was confirmed by mass spectrometry. The detection limit of this method for the diols is 1 nanogram (11 picomoles).

RESULTS

Ethanol in concentrations ranging from 3 to 96 μM was found in the serum of 78 of the 113 alcoholics but in none of the 54 controls.

2,3-butanediol was found in concentrations ranging from 0.01 to 0.841 μM in the serum of 79 of the 113 alcoholics tested at the time of admission to the alcohol unit. The serum of 10 alcoholics contained no measurable amount of ethanol, but nevertheless contained 2,3-butanediol in concentration ranging up to 0.059 μM. The serum of 9 of the 113 alcoholic subjects had ethanol in concentrations as high as 85 μM,

but no detectable 2,3-butanediol. None of the samples of patients or controls contained 1,3-butanediol.

Regression analysis showed no correlation between the concentration of ethanol and of 2,3-butanediol in the blood at the time of admission but the serum of the 26 patients on whom second blood samples drawn 18 hours after admission contained no measurable amount of ethanol and, in each case, 2,3-butanediol had virtually disappeared.

In addition to ethanol and 2,3-butanediol the presence of 1,2-propanediol was also detected. Unfortunately, because many of the specimens were collected in vacutainers lined with a plasticizer that apparently contained traces of 1,2-propanediol, the values for 1-2, propanediol listed in Table 1 may be high except when the specimens were collected in clean glass. Contamination must be slight, however, since at the time of admission the serum of patients 83, 90 and 95 contained 1,2-propanediol while 1,2-propanediol was absent from specimens collected 18 hours later in the same type of vacutainer. The measurement of 2,3-butanediol was unaffected by the vacutainer plasticizer as shown by the absence of 2,3-butanediol in all of the control serums drawn in vacutainers as well as glass.

The clinical diagnoses made entirely independently of this study included alcoholic hepatitis or cirrhosis of the liver in 29 of the alcoholic subjects; diabetes mellitus in 8 and Korsakoff's syndrome in 6. (See Table 1). There was no correlation between either the presence or severity of these conditions and the presence or absence of butanediol in the blood. Neither did the concentration of butanediol correlate

with the age of the patient, severity or duration of the alcoholism, or the type of alcoholic beverage said to be consumed. Indeed, the groups with and without 2,3-butanediol in their blood although discordant as to numbers, were well matched on age and sex as well as with respect to the above clinical data.

A diagnosis of mental depression had been recorded on the hospital charts of 22 (20%) of the 113 alcoholic subjects. The serum of all but 2 contained measurable concentration of ethanol ranging from 4 to 70 μM. 2,3-butanediol in concentration ranging from 0.13 to 0.14 was found in the serum of all but one of them -- a 24 year old woman -- one of the two whose serum contained no ethanol, and who according to the history had been two days without any alcohol intake.

DISCUSSION

The source of the 2,3-butanediol found in the blood of the majority of unselected alcoholics in this study cannot be stated at this time, although the reduction of acetoin to 2,3-butanediol is a well known pathway of microbial metabolism and has also been shown to occur *in vivo* (Dawson and Hullin, 1954). Acetoin has been produced *in vivo* following acetaldehyde ingestion (Stotz, et al., 1944). Recently Veech and collaborators demonstrated the production of 2,3-butanediol in germ-free rats during the metabolism of ethanol. Moreover, they demonstrated acetoin production by the brain and 2,3-butanediol formation from acetoin by the liver (Veech, et al., 1981). The clinical significance of the presence of 2,3-butanediol in the blood of alcoholics remains to be determined. The absence of 2,3-butanediol in a segment

of the population of alcoholics (12%) despite high serum concentrations of ethanol suggests the possibility of a genetic basis for the high alcohol (Rutstein and Veech, 1978). The suggestive association of the presence of 2,3-butanediol in alcoholics with an independently made diagnosis of depression is intriguing but inconclusive. It does, however, suggest the need for a thorough study of the relationship of 2,3-butanediol to brain function in alcoholic subjects.

SUMMARY

Blood was drawn from 113 alcoholic subjects admitted to a detoxification center and from 54 non-alcoholic controls including 7 diabetic patients. Ethanol was present in the blood of 78 (69%) of the alcoholic subjects and none of the controls. Among the 78 alcoholic subjects in whom ethanol was identified in the blood, 69 or 88% also had 2,3-butanediol in their blood. In 9 or 12% of the 80, 2,3-butanediol was not present in detectable amounts despite ethanol levels as high as 85 μM. Among the 35 alcoholics whose blood contained no ethanol but nearly all of whom had acknowledged consuming alcohol within the past 24 hours, 10 (28%) had detectable amounts of 2,3-butanediol in their blood in concentration varying from 0.011 to 0.059 μM.

Thus, while butanediol concentrations did not correlate with the concentration of ethanol in the blood, its presence, nevertheless, appeared to depend on recent ethanol ingestion since the butanediol had greatly decreased or disappeared from the blood of those patients retested 18 hours after admission. There was no osbserved correlation between age, sex, evidence of liver damage, diabetes, or evidence of

Korsakoff's syndrome and the presence or absence of 2,3-butanediol. There was, however, a suggestive correlation with independently gathered evidence of mental depression. While the significance of the presence of 2,3-butanediol is unknown at this time, that data do suggest the need for a careful investigation of the relationship of 2,3-butanediol to brain function.

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TABLE 1

Data on 113 Unselected Patients Admitted to an Alcohol Detoxification Center. Blood Specimens Collected in Glass are Identified by * in the 1,2-propanediol column

NUMBER	INITIALS	AGE	SEX	ETHANOL µM	2,3 BUTANEDIOL µM	1,2 PROPANEDIOL µM	ASSOCIATED ABNORMALITIES
88	J.D.	62	M	74	0.841	0.19	Gamma GT 268, Amylase 93 SGPT 16
73	W.A.	57	M	11	0.775	0.09	Hepatitis, Gamma GT 462 SGPT 22
53	C.H.W.	50	M	53	0.655	0.20	Hepatitis, Gamma GT 305 SGPT 34
56	J.C.	32	M	96	0.255	0.10	
179	R.L.	-	M	80	0.201	0.03*	
67a	E.K.	44	M	43	0.158	0.15	
b				0	<0.01	0.12	
54	J.A.H.	46	M	74	0.144	0.17	Hepatitis
48a	J.M.	50	M	58	0.141	0.16	
b				0	<0.01	0.12	
14	W.R.	51	M	19	0.140	0.45	Depression
50a	J.H.	50	M	35	0.137	0.95	Korsakoff's, Cirrhosis, Diabetes, Depression, Gamma GT 2490, SGPT 37
b				0	<0.01	0.09	
6	W.D.	47	F	61	0.122	0.14	Depression
15	L.N.	41	M	64	0.121	0.19	Hepatitis & Korsakoff's
7	G.B.	45	M	36	0.118	0.1[illegible]	Polyneuritis, antibuse toxicity, Depression, Hepatitis, Gamma GT 191 SGPT 35
63	M.L.	20	M	48	0.118	0.46	
12	E.W.	32	M	75	0.112	0.35	Korsakoff's, Hepatitis, Gamma GT 237, SGPT 25
66a	S.O.S.	57	M	51	0.109	0.11	Hepatitis & Cirrhosis
b				0	<0.01	0.05	
13	M.T.	35	M	21	0.106	0.37	
52a	D.S.	45	M	54	0.100	0.19	Hepatitis, Cirrhosis, Malnutrition
b				0	0.012	0.06	
8	H.B.	60	M	34	0.099	0.16	Hepatitis, Gamma GT 133

TABLE 1

NUMBER	INITIALS	AGE	SEX	ETHANOL µM	2,3 BUTANEDIOL µM	1,2 PROPANEDIOL µM	ASSOCIATED ABNORMALITIES
4	J.A.M.	47	M	45	0.097	0.13	Depression
193	J.O.	45	M	48	0.096	0.07*	
16	W.F.C.	49	M	42	0.095	0.17	Depression
75	E.S.B.	47	M	68	0.093	0.18	Hepatitis, Depression
64a	D.U.	39	M	31	0.087	0.17	
b				0	0.014	0.14	
20	H.F.	58	M	43	0.086	0.16	Depression, suicide attempt
104	W.F.	39	M	42	0.082	0.16	
189	B.P.	38	F	39	0.080	<0.01*	
103	C.C.	57	M	42	0.078	0.14	SGPT 50
10	D.F.	49	M	24	0.078	0.018	Hepatitis, Gamma GT 249
71	J.E.M.	25	M	20	0.077	0.17	
23	D.B.	34	F	45	0.072	0.12	Depression with suicidal ideation
3	J.S.	42	F	50	0.072	0.16	
25	O.C.	53	F	70	0.071	0.44	Depression, Gamma GT 183 SGPT 19
82a	F.N.	33	F	9	0.064	0.11	
b				0	<0.01	0.13	
87	R.R.	27	M	51	0.063	0.10	
122	A.A.	47	M	53	0.062	0.15	
200	R.M.H.	56	F	48	0.06	0.10	Depression
76	J.L.R.	35	M	0	0.059	0.14	Diabetes, Depression, Gamma GT 601 SGPT 67
62	S.W.S.	61	F	46	0.053	0.20	Depression
41	M.H.B.	50	M	0	0.052	0.17	Gamma GT 276, SGPT 30
191	J.W.	31	F	19	0.050	0.02*	

TABLE 1

NUMBER	INITIALS	AGE	SEX	ETHANOL µM	2,3 BUTANEDIOL µM	1,2 PROPANEDIOL µM	ASSOCIATED ABNORMALITIES
42	H.S.	55	M	27	0.048	0.40	Hepatitis, Gamma GT 154 SGPT 30
5	R.B.	38	M	61	0.044	0.66	Diabetes, Depression with suicide attempt
26	W.P.	38	M	56	0.041	0.10	Hepatitis, Cirrhosis, Gamma GT 439, SGPT 14
22	F.P.	30	M	43	0.040	0.13	Violent behavior
192	C.A.K.	22	F	56	0.040	<0.01*	
84a	A.D.	38	M	58	0.039	0.16	Gamma GT 930, SGPT 17
b				0	<0.01	0.14	
190	B.S.	52	F	3	0.039	<0.01*	
38	W.E.C.	51	M	49	0.036	0.13	Depression
2	J.P.	65	M	4	0.036	0.23	
58	L.B.H.	30	F	74	0.035	0.13	Gamma GT 84, SGPT 32
178	G.R.W.	33	F	0	0.035	0.08*	Gamma GT 229, SGPT 28
55	M.S.	31	F	43	0.034	0.18	
70a	J.P.S.	51	M	40	0.033	0.17	
b				0	<0.01	0.08	
83a	B.R.	53	M	46	0.033	0.30	Gamma GT 87, SGPT 40
b				0	<0.010	<0.01	
9	S.F.	30	M	11	0.031	0.16	Depression, Hepatitis, Gamma GT 213, SGPT 32
68	W.S.	41	M	0	0.030	0.12	
198	C.C.	58	M	38	0.030	0.14*	
80	S.S.S.	37	M	0	0.018	0.06	Gamma GT 101, SGPT 51
29	H.W.	15	M	31	0.017	0.15	Hepatitis
30	M.W.B.	51	F	53	0.017	0.18	Depression, Hepatitis Gamma GT 92, SGPT 52
99	W.K.	55	M	47	0.016	0.10	Gamma GT 7800, SGPT 61

TABLE 1

NUMBER	INITIALS	AGE	SEX	ETHANOL µM	2,3 BUTANEDIOL µM	1,2 PROPANEDIOL µM	ASSOCIATED ABNORMALITIES
74	R.O.D.	46	M	4	0.015	0.05	Hepatitis, Cirrhosis, Depression
69a	C.W.	51	M	53	0.013	0.34	Hepatitis, Gamma GT 252, SGPT 43
b				0	<0.010	0.14	
186	G.W.	53	M	24	0.013	<0.01*	Depression
11	L.H.	32	F	47	0.012	0.15	Hepatitis, Gamma GT 148
72a	H.G.	36	M	47	0.011	0.41	Diabetes
b				0	<0.010	0.22	
17	J.G.	33	M	71	<0.01	0.22	
18	W.J.	57	M	29	<0.01	0.22	Hepatitis
21	D.R.	46	M	0	<0.01	0.14	Hepatitis, Gamma GT 850, SGPT 48
24	M.J.B.	47	F	85	<0.01	0.21	Diabetes, Korsakoff's, Hepatitis Gamma GT 129, SGOT 48
27	W.N.	36	M	0	<0.01	0.18	S, SA
44a	J.A.C.	47	M	9	<0.01	0.16	
b				0	<0.01	0.11	
180a	A.G.	36	M	40	0.030	0.13	Gamma GT 193, SGPT 29
b				0	0.023	0.05	
51a	F.B.	22	M	28	0.029	0.14	
b				0	<0.01	0.12	
77	J.N.S.	52	M	0	0.027	0.11	
57	J.J.S.	45	M	47	0.025	0.35	Korsakoff's, Depression SGPT 39
97a	C.S.	73	F	46	0.024	0.10	Congestive failure
b				37	0.014	0.15	
194	D.C.	20	M	0	0.024	0.13	

TABLE 1

NUMBER	INITIALS	AGE	SEX	ETHANOL µM	2,3 BUTANEDIOL µM	1,2 PROPANEDIOL µM	ASSOCIATED ABNORMALITIES
46a	H.D.W.	51	M	0	0.022	0.30	Hepatitis, Depression, SGPT 38
b				0	0.10	0.15	
19	F.H.	38	M	39	0.021	0.05	Hepatitis, Cirrhosis, Diabetes, Gamma GT 3240, SGPT 95
182	J.B.	63	M	4	0.020	<0.01*	
181	T.Q.	29	M	0	0.020	0.08*	
196a	A.T.	46	F	0	0.020	<0.01*	
b				0	<0.010	<0.01*	
59	G.M.	51	M	64	0.018	0.17	Gamma GT 154, SGPT 17
47	J.U.	33	F	0	<0.01	0.10	Diabetes, Hepatitis, Gamma GT 212, SGPT 48
60a	J.J.M.	40	M	24	<0.01	0.27	Hepatitis, Gamma GT 210, SGPT 46
b				0	<0.01	0.08	
61	A.M.	30	M	0	<0.01	0.08	Hepatitis
65a	V.B.	25	F	0	<0.01	0.17	
b				0	<0.01	0.14	
79a	G.W.M.	65	M	0	<0.01	0.13	Gamma GT 93, SGPT 26
b				0	<0.01	0.12	
81	A.A.	51	M	0	<0.01	0.09	Schizophrenia
85a	W.G.	67	M	0	<0.01	0.21	Proteins & Ketones in Urine Gamma GT 340, SGPT 58
b				0	<0.01	0.11	
86a	W.H.	67	M	0	<0.01	0.15	
b				0	<0.01	0.12	
89	J.B.H.	70	M	0	<0.01	<0.01*	
92	J.S.	37	M	34	<0.01	0.18	Ketoneuria, Gamma GT 95, SGPT 31

TABLE 1

NUMBER	INITIALS	AGE	SEX	ETHANOL µM	2,3 BUTANEDIOL µM	1,2 PROPANEDIOL µM	ASSOCIATED ABNORMALITIES
93a	N.H.A.	64	M	5	<0.01	0.19	Diabetes, Korsakoff's Gamma GT 15, SGPT 51
b				0	<0.01	0.05	
94	E.S.	50	M	0	<0.01	0.04	Hepatitis, Diabetes
95a	D.J.F.	28	M	0	<0.01	0.07	Gamma GT 247
b				0	<0.01	<0.01	
96	E.A.S.	43	M	0	<0.01	0.07	Gamma GT 217, SGPT 29
98	E.D.	79	F	0	<0.01	0.05	
100	W.A.F.	22	M	0	<0.01	0.05	Gamma GT 84, SGPT 35
101	R.F.	31	M	0	<0.01	0.10	
102	C.K.	22	F	0	<0.01	0.08	Ketonuria, Gamma GT 11, SGPT 41
105	B.S.	24	F	0	<0.01	0.14	Depression, suicide attempt, drug abuse, Gamma GT 6.8, SGPT 71
90a	G.W.	50	F	0	<0.01	0.05	
b				0	<0.01	<0.01	
91	D.S.	64	M	0	<0.01	0.03	
45	S.W.	31	M	0	<0.01	0.33	
78a	M.J.S.	24	M	0	<0.01	0.07	
b				0	<0.01	0.07	
1	B.G.	38	F	69	<0.01	0.13	
183	M.L.	67	F	21	<0.01	*	
184	N.C.	70	M	0	<0.01	*	
188	L.M.	33	M	0	<0.01	<0.01*	
195	G.S.	29	M	0	<0.01	<0.01*	


ATE
LME
8