Venous blood ammonium levels in control subjects and in patients with disorders of the liver

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SYNOPSIS Venous blood ammonium levels were studied in 106 control subjects and 47 patients with varying degrees of liver disorder. The resting venous blood ammonium was normally distributed in both male and female control subjects and was not influenced by either the sex or the fasting state of the subject. In general it was also uninfluenced by age except that the level was found to be a little lower in subjects over 60 years of age probably due to their state of greater muscular inactivity.

The mean resting ammonium level in controls was $80.0 \mu\text{g}/100 \text{ ml} \pm 17.17 \mu\text{g}/100 \text{ ml}$ and the range (mean $\pm 2 \text{ SD}$) $46\text{--}114 \mu\text{g}/100 \text{ ml}$. Raised levels were obtained in 16.7% of patients with subclinical liver disorder, 62.5% with moderate liver disorder, and 85.5% with severe liver disorder, indicating a relationship between the severity of liver disorder and the resting venous blood ammonium level. As the majority of patients with severe liver disorder were known to have varices the raised ammonium levels are likely to have been related to the greater magnitude and incidence of portal systemic shunts in those with severe liver disease.

The estimation of blood ammonium, although still largely a research investigation, may give information of considerable clinical importance. The relationship of ammonium to hepatic coma remains an enigma but despite occasional failure to find a correlation few investigators would be prepared to conclude that none exists.

Hitherto, errors caused by rapid decomposition of shed blood and by artefacts in ammonium determination have often invalidated the clinical significance of blood ammonium estimations (Perea and Nelson, 1964). Controversy exists as to how much of the ammonium removed from the blood represents preformed ammonium and how much is the result of decomposition of shed blood before and during analysis (Conway, 1935; Bromberg, Robin, and Forkner, 1960; Zieve, 1966). There has been no consistent agreement on the normal range of blood ammonium nor attention paid to the influence of possible

modifying factors such as age, sex, and the fasting or resting state of the subject.

The direct colorimetric method of determining blood ammonium concentration described by McCullough (1967) is a modification of the technique of Okuda, Fujii, and Kawashima (1965) and provides a simple and reliable method of simultaneously estimating the ammonium content of a number of blood samples thus saving technicians' time. Atmospheric contamination, important in the diffusion technique, is not a problem and elaborate equipment is not required. Furthermore, the results are readily reproducible with a coefficient of variation of 1%.

Because of the obvious advantages offered by this technique, the time seemed opportune (1) to establish values for blood ammonium in apparently healthy subjects resident in the Belfast area of Northern Ireland, and (2) to study the relationship between the severity of hepatic disorders assessed by clinical and biochemical criteria and the resting and fasting venous blood ammonium concentration.

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Material and Methods

A total of 106 control subjects and 47 patients with hepatic disorders took part in the present investigation. The control subjects were taken from various social classes and included doctors, nurses, medical students, laboratory personnel, secretarial staff, members of the armed forces, domestic servants, and labourers, none of whom was known to be suffering from hepatic, cardiac, or pulmonary diseases liable to influence the blood ammonium levels (Bessman and Evans, 1955; Dutton, Nicholas, Fisher, and Renzetti, 1959).

It has been shown that muscular activity can cause considerable increases in venous blood ammonium concentration (Allen and Conn, 1960; Sinniah, Fulton, and McCullough, 1970) and this effect can be largely counteracted by ensuring that the subjects rest for at least 30 minutes before the blood samples are withdrawn for analysis. For this reason both control subjects and patients with liver disorder were made to rest completely for a minimum period of 30 minutes before blood sampling.

Resting blood ammonium levels were obtained in 46 male and 40 female control subjects while the remaining 13 male and seven female subjects had also fasted for 12 hours. The control subjects were allocated into groups according to sex, age (20-39, 40-59, 60-79 years), and their state of satiety.

The 47 patients with liver disorders included 33 with cirrhosis, nine with chronic hepatitis, four recovering from acute hepatitis, and one with healed hepatic abscess. They were carefully examined and allocated into three groups according to their hepatic status.

GROUP 1: SUBCLINICAL LIVER DISORDER
Patients with no demonstrable clinical signs of liver disease and in whom the diagnosis depended upon the past history and biochemical abnormality. There were 12 patients in this group, two with cirrhosis, five with chronic hepatitis, four recovering from acute hepatitis, and one with healed hepatic abscess. The mean age of these patients was 33.8 years and the range 19-60 years.

GROUP 2: MODERATE LIVER DISORDER
Patients with both clinical signs and biochemical abnormalities of liver disease, but who had neither the clinical manifestations nor any previous history of hepatic encephalopathy. There were 16 patients in this group, 12 with cirrhosis and four with chronic hepatitis. Their mean age was 50.8 years and the range 30-67 years.

GROUP 3: SEVERE LIVER DISORDER
Patients with clinical and biochemical indications of liver disease who had either been in pre-coma or coma immediately before the period of investigation, subsequently developed such a disorder or showed persistent signs of hepatic encephalopathy. This group comprised 19 cirrhotic patients whose ages ranged from 32 to 72 years, with a mean of 56.3 years. That patients in group 3 were generally older than those less severely affected is probably an indication that in them the disease was of longer standing and greater progression.

Using the direct colorimetric method described by McCullough (1967) venous blood ammonium levels were determined in all cases with the subjects resting after a 12-hour fast. The results are expressed in micrograms of ammonium nitrogen/100 ml blood.

Results

The mean resting, non-fasting venous blood ammonium level in 86 control subjects was 80 $\mu\text{g}/100\text{ ml}$ (SD 17.17 $\mu\text{g}/100\text{ ml}$) and 95% of the ammonium levels ranged between 46 and 114 $\mu\text{g}/100\text{ ml}$. There was no appreciable difference in the ammonium levels between male and female subjects (mean values of 80.26 and 79.68 $\mu\text{g}/100\text{ ml}$ respectively). The frequency distribution of the resting blood ammonium level in the 86 male and female controls is shown in Figure 1.

FREQUENCY DISTRIBUTION OF VENOUS BLOOD AMMONIUM WITH REGARD TO SEX

In males

The frequency distribution of blood ammonium in the 46 resting male controls is shown in Figure

Fig. 1 Frequency distribution of resting venous blood ammonium in normal subjects.

2. The histogram does not appear to show any marked skewness. A statistical analysis was done to determine if there was a normal distribution of venous blood ammonium in the male subjects. The results for skewness and kurtosis with infinite degrees of freedom were $0.2 > P > 0.1$ and $0.9 > P > 0.8$ respectively. These values are not significant at $P = 0.05$.

In females

The frequency distribution of blood ammonium in the 40 resting female controls is shown in Figure 3. The values for measuring skewness and kurtosis with infinite degrees of freedom were $0.5 > P > 0.4$ and $0.4 > P > 0.3$ respectively.

Fig. 2 Frequency distribution of resting venous blood ammonium in normal male subjects.

Fig. 3 Frequency distribution of resting venous blood ammonium in normal female subjects.

Neither of these values were significant at $P = 0.05$. There was therefore a normal distribution of venous blood ammonium in the females as in the males.

THE INFLUENCE OF AGE ON THE RESTING BLOOD AMMONIUM LEVEL

In males

The relationship of venous blood ammonium to age in the 46 resting male controls is shown in the scattergram (Fig. 4). The mean ammonium value was $82.2 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 14.8 \mu\text{g}/100 \text{ ml}$) in controls aged 20-39 years, $86.7 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 14.0 \mu\text{g}/100 \text{ ml}$) in controls aged 40-59 years, and $67.8 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 17.2 \mu\text{g}/100 \text{ ml}$) in controls aged 60-79 years (Table I).

It was found that there were no significant differences between the mean ammonium concentrations of the three groups of subjects: variance ratio $F = 3.2$ and at degrees of freedom $n_1 = 2, n_2 = 43, P > 0.05$.

Age (yr)	No. of Observations	Resting Blood Ammonium Level ($\mu\text{g}/100 \text{ ml}$)		
		Range ¹	Mean	SD
20-39	28	52.6-111.8	82.2	14.8
40-59	9	58.7-114.7	86.7	14.0
60-79	9	33.4-102.2	67.8	17.2

Analysis of variance $P > 0.05$

Table I Resting venous blood ammonium levels in males aged 20-39, 40-59, and 60-79 years

¹Range = mean $\pm$ 2 SD

In females

The relationship of the resting venous ammonium level to age in the 40 female controls is shown in the scattergram (Fig. 5). The mean ammonium level was $83.9 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 15.6 \mu\text{g}/100 \text{ ml}$) in controls aged 20-39 years, $82.0 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 16.7 \mu\text{g}/100 \text{ ml}$) in controls aged 40-59 years, and $70.8 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 15.4 \mu\text{g}/100 \text{ ml}$) in controls aged 60-79 years (Table II). An analysis of variance revealed no significant differences between the mean ammonium concentrations of the resting subjects belonging to the

Age (yr)	No. of Observations	Resting Blood Ammonium Level ($\mu\text{g}/100 \text{ ml}$)		
		Range ¹	Mean	SD
20-39	22	52.7-114.1	83.9	15.6
40-59	6	48.6-115.4	82.0	16.7
60-79	12	40.0-101.6	70.8	15.4

Analysis of variance $P > 0.05$

Table II Resting venous blood ammonium levels in females aged 20-39, 40-59, and 60-79 years

¹Range = mean $\pm$ 2 SD

Fig. 4 Scattergram showing the relationship of resting venous blood ammonium to age in normal males.

Fig. 5 Scattergram showing the relationship of resting venous blood ammonium to age in normal females.

Group	Standard Error of Difference (µg/100 ml)	P = 0.05	Minimum Significant Difference (µg/100 ml)	Difference in Means (µg/100 ml)	Probability at P = 0.05
B-A	4.816	2.00	9.632	1.84	Not significant
B-C	5.53	2.00	11.06	15.32	Significant
C-A	4.2536	2.00	8.5072	-13.48	Significant

Table IV Analysis to determine the significance of the difference in the means between the control groups

A = controls aged 20-39 years
B = controls aged 40-59 years
C = controls aged 60-79 years

different age groups: variance ratio $F = 2.79$ and at degrees of freedom $n_1 = 2$, $n_2 = 37$, $P > 0.05$, which is not significant.

When the resting venous blood ammonium levels of both male and female controls were grouped collectively according to age groups, it was found that there was no significant difference between the mean blood ammonium level of the controls aged 20-39 years and those aged 40-59 years. The mean blood ammonium level of the controls aged 60 years and over was lower than that in the younger controls (Tables III and IV).

THE INFLUENCE OF SEX ON THE RESTING VENOUS AMMONIUM LEVEL

t Tests were undertaken to determine if there were significant differences between the mean blood ammonium concentrations of male and female controls belonging to the age groups 20-39, 40-59, and 60-79 years. It was found that there were no significant differences between the mean ammonium concentration of male and female controls. For the three groups $t = 0.35$, $t = 0.59$, and $t = 0.42$, and at 48, 13, and 19 degrees of freedom respectively, $0.8 > P > 0.7$, $0.6 > P > 0.5$, and $0.7 > P > 0.6$, which are not significant at $P = 0.05$.

THE INFLUENCE OF FASTING ON THE RESTING VENOUS AMMONIUM LEVEL

The ammonium levels of 20 fasting control subjects (13 male and seven female) aged 20-39 years were compared with those of 50 non-fasting controls (28 male and 22 female) of the same age group. The mean ammonium concentrations in the fasting and non-fasting controls were $86.9 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 15.5 \mu\text{g}/100 \text{ ml}$ and range $55.9-117.9 \mu\text{g}/100 \text{ ml}$) and $82.9 \mu\text{g}/100 \text{ ml}$ ($\text{SD} \pm 16.8 \mu\text{g}/100 \text{ ml}$ and range $49.3-116.5 \mu\text{g}/100 \text{ ml}$) respectively. t Tests (Table V) show no significant difference between the mean blood ammonium concentrations of fasting and non-fasting controls. It was also found that there is no significant difference between the 'scatter' of the blood ammonium levels in the two groups of subjects, scatter $F = 1.17$ and at $n_1 = 49$, $n_2 = 19$ degrees of freedom, $P > 0.05$, which is not significant.

Group	No. of Observations	Resting Blood Ammonium Level (µg/100 ml)		
		Mean	SE	SD
A	50	82.96	2.31	16.80
B	15	84.80	4.22	14.74
C	21	69.48	3.57	15.90

Analysis of variance $0.05 > P > 0.01$

Table III Mean resting venous blood ammonium level in controls (86 males and females) aged 20-39, 40-59, and 60-79 years

A = controls aged 20-39 years
B = controls aged 40-59 years
C = controls aged 60-79 years

SE = standard error

SD = standard deviation

Neither satiety nor fasting influenced the resting venous blood ammonium concentration in the control subjects.

THE RELATIONSHIP BETWEEN THE SEVERITY OF LIVER DISORDER AND THE FASTING VENOUS BLOOD AMMONIUM LEVEL

Figure 6 is a scattergram of the fasting venous blood ammonium levels in control subjects, and in patients with mild, moderate, and severe liver disorders, and Table VI shows the mean values and their standard deviations. Control subjects were found to have a mean fasting venous blood ammonium level of 86.9 $\mu\text{g}/100\text{ ml}$ (SD $\pm 15.5\text{ }\mu\text{g}/100\text{ ml}$) while patients with mild or subclinical

liver disorder were found to have a mean ammonium level of 79.1 $\mu\text{g}/100\text{ ml}$ (SD $\pm 27.76\text{ }\mu\text{g}/100\text{ ml}$). The mean ammonium level in patients with moderate liver disorder was 125.0 $\mu\text{g}/100\text{ ml}$ (SD $\pm 22.04\text{ }\mu\text{g}/100\text{ ml}$) while patients with severe liver disorder had a mean ammonium level of 172.2 $\mu\text{g}/100\text{ ml}$ (SD $\pm 62.0\text{ }\mu\text{g}/100\text{ ml}$).

Statistical analysis was used to determine if there were significant differences between the mean venous blood ammonium levels in the four groups of subjects. It was found that the variance ratio $F = 21.84$ and at $n_1 = 3, n_2 = 63$ degrees of freedom, $p < 0.01$ which is significant at $p = 0.05$. Calculation of the minimum significant difference between the means of the different groups showed that although there was no significant difference between the mean venous blood ammonium level of control subjects and patients with subclinical liver disorder, there were (Tables VI and VII) highly significant differences

Mean Blood Ammonium ($\mu\text{g}/100\text{ ml}$)		Mean Difference ($\mu\text{g}/100\text{ ml}$)	Standard Error of Difference ($\mu\text{g}/100\text{ ml}$)	Degrees of Freedom	
Resting	Resting				
Non-fasting	Fasting				
82.9 (50) ¹	86.9 (20) ¹	4.0	4.4	$t = 0.91$	68
$0.4 > p > 0.3$					

Table V Use of *t* test to determine any significant difference in venous blood ammonium levels of fasting resting and non-fasting resting subjects aged 20-39 years

¹Number of subjects studied in the respective groups

Group	No. of Observations	Fasting Blood Ammonium Level ($\mu\text{g}/100\text{ ml}$)			
		Range ¹	Mean	SE	SD
A	20	55-108	86.9	8.3	15.5
B	12	52-137	79.1	10.94	26.76
C	16	89-167	125.0	9.47	22.04
D	19	82-357	172.21	8.69	62.0
Analysis of variance $p > 0.01$					

Table VI Fasting venous blood ammonium levels in subclinical, moderate, and severe liver disorder groups and in control group

¹Range - from the lowest to the highest recorded value

- Group A = control
- Group B = subclinical liver disorder
- Group C = moderate liver disorder
- Group D = severe liver disorder

Group	SE of Difference ($\mu\text{g}/100\text{ ml}$)	$P = 0.05$	MSD ($\mu\text{g}/100\text{ ml}$)	Difference in Means ($\mu\text{g}/100\text{ ml}$)
B-A	13.0	2.0	26.0	6.9
C-A	12.75	2.0	25.5	40.43 ¹
D-A	11.96	2.0	23.92	85.31 ¹
C-B	13.87	2.0	27.74	47.33 ¹
D-B	13.15	2.0	16.3	92.21 ¹
D-C	12.90	2.0	25.8	44.88 ¹

Table VII Analysis to show the significance of the difference in the means between the groups

¹Significant at 0.05 probability

- A = control
 - B = subclinical liver disorder
 - C = moderate liver disorder
 - D = severe liver disorder
- MSD = minimum significant difference

Fig. 6 Scattergram showing the fasting resting venous blood ammonium values in the different groups of liver disorder.

between the mean venous blood ammonium levels of (1) controls and patients with moderate liver disorder; (2) controls and patients with severe liver disorder; (3) patients with subclinical liver disorder and patients with moderate liver disorder; (4) patients with subclinical liver disorder and patients with severe liver disorder; and (5) patients with moderate liver disorder and patients with severe liver disorder.

All the 20 fasting controls had ammonium levels which fell within the range established earlier in 86 resting non-fasting male and female control subjects (normal range 46-114 $\mu\text{g}/100\text{ ml}$): 16.7% of patients with subclinical liver disorder, 62.5% of patients with moderate liver disorder, and 89.5% of patients with severe liver disorder had blood ammonium levels exceeding 114 $\mu\text{g}/100\text{ ml}$.

Discussion

The present study shows that the resting venous blood ammonium level is normally distributed in both male and female controls. Although there are no significant differences between the mean ammonium levels of subjects aged 20-39 and 40-59 years, the mean ammonium level of controls aged 60 years and over appears to be a little lower than that of younger subjects. This may be due to the state of greater muscular inactivity of the older subjects.

No significant differences were found between either the means or the scatter of the venous blood ammonium levels in fasting and non-fasting controls, indicating that in them no significant elevation of blood ammonium occurred in response to a normal meal containing protein. Nielsen, Liang, and Chey (1965) reported similar findings in their control subjects after a protein meal.

The normal resting venous blood ammonium level was found to be between 46 and 114 $\mu\text{g}/100\text{ ml}$, differing little from the range 48-113 $\mu\text{g}/100\text{ ml}$ reported by McCullough (1967). Previous workers using other methods of analysis had obtained very variable normal venous blood ammonium levels (Table VIII) making comparison of results impossible.

The present study shows a direct relationship between the severity of the liver disorder and the incidence and height of elevated blood ammonium levels, ie, 16.7% of those with subclinical liver disorder, 62.5% of patients with moderate liver disorder, and 89.5% of patients with severe liver disorder had raised venous blood ammonium levels. Phear, Sherlock, and Summerskill (1955) found normal blood ammonium levels in 10% of their patients with hepatic encephalopathy and this compares closely with our figure of 10.5%. Phear *et al* (1955) discussed the confusion caused by the finding of a normal blood ammonium level in occasional cases of coma and of high ammonium levels in the absence of neuropsychiatric disturbance

The majority of patients in the 'severe liver disorder group' were known to have varices (though detailed investigation was not undertaken in every case) and this finding is in accord with and supports the present consensus of opinion that the blood ammonium level is a measure of portal-systemic shunting (Fenton, 1967). Furthermore, the 10.5% of patients in the 'severe liver disorder group' with normal blood ammonium levels may well have had severely impaired liver cell function rather than any significant portal-systemic shunting.

Susceptibility to hepatic coma has been thought to be directly related to the severity of impairment of liver function, the magnitude of the portal-systemic collateral circulation, and the amount of a toxic factor formed in the intestine. The greater the latter, the less severe need be hepatocellular dysfunction and the smaller the portal-systemic collateral circulation before cerebral manifestations appear (Zieve, 1966). Thus, the main cause of hepatic coma is not the same in all cases, and McDermott, Wareham, and Riddell (1955) distinguished between 'endogenous' and 'exogenous' varieties. The latter, with an almost invariably raised blood ammonium level, corresponds to the toxic intestinal cause, 'enterogenous coma', and often has a better prognosis than the spontaneous or 'endogenous' type with its basis in progressive parenchymal failure (Stahl, 1963). The blood ammonium level in this type of coma may be normal.

The blood ammonium level may not bear a strict relationship to encephalopathic symptoms at the time of sampling, and it has been suggested that it 'anticipates' the subsequent course of events, the maximum concentration being reached before coma is fully developed (Egense, 1960 and 1963). An increase in the blood ammonium level often precedes the onset of symptoms and a fall heralds recovery (Stahl, 1963; Fenton, 1965). Whereas sharp but transient rises in blood

Author	Blood Ammonia Nitrogen Range ($\mu\text{g}/100\text{ ml}$)
Barnett (1917)	0- 50
Stanojević (1931)	17- 37
Conway (1935)	0- 38
Kirk (1936)	18- 40
McDermott and Adams (1954)	50- 79
Seligson and Hirahara (1957)	75-196
Egense (1960)	46-134
Burg and Mook (1963)	10- 70
Mondzac, Ehrlich, and Seegmiller (1965)	17- 80 ¹
Okuda <i>et al</i> (1965)	100-150
McCullough (1967)	48-113
Present study	46-114

Table VIII Comparison of 'normal' venous blood ammonium results with values in other published reports

¹Plasma nitrogen $\mu\text{g}/100\text{ ml}$

ammonium concentration may cause no untoward effects a sustained increase may be followed by the appearance of the clinical syndrome of hepatic encephalopathy.

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