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THE ROLE OF VITAMIN C DEFICIENCY
IN CHRONIC ALCOHOLISM

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A Thesis submitted for the degree of Master of Science
of the University of Glasgow.

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I would like to express my gratitude to Professor A. Goldberg for his guidance and encouragement during the course of my research project.

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I am indebted to the Medical Council for Alcoholism for the Grant provided.
SUMMARY

Gastric erosions caused by home-brewed lager.

A 22 year old male was admitted to hospital because of melaena due to acute gastric erosions. In the three weeks prior to his admission, he had drunk several gallons of home-brewed lager. Investigations revealed that this was contaminated with acetaldehyde and acetic acid which had been produced by an acetobacter organism present in the yeast. Experiments were carried out on the effect of home-brewed lager and beer on the stomachs of the guinea pigs. The incidence of erosive gastritis was significantly increased when the animals were given similarly contaminated home-brewed lager. This observation is of importance since the home-brewing of beer and lager has become common since its legalisation in 1964.

A clinical study was carried out to determine the leucocyte ascorbic acid levels and the iron status of fifty alcoholics admitted to medical and psychiatric wards. In addition, the dietary intake of vitamin C and iron was estimated.

The age range of the group was 24-70 years and this included nine females, age range 34-50 years. None of the patients had overt liver disease and none were intoxicated at the time the blood sample was taken.

All the alcoholic patients had an inadequate intake of iron (15 mg/day) but the haemoglobin, erythrocyte
protoporphyrin and serum iron levels showed that over 55% of the patients showed no signs of anaemia. In the absence of liver disease, the incidence of anaemia in alcoholics was lower than expected.

L.A.A. was estimated by the 2,4 dinitrophenyl method and the average daily intake of vitamin C was calculated from the diet history. The alcoholic group was matched with a normal group of patients according to age (within five years) and sex. The results show that, as a group, alcoholics are sub-clinically scorbutic. Their vitamin C levels, \(18.8 \pm 11.01\) (mean \(\pm\) S.D.) \(\mu g/10^8\) WBC are significantly lower (\(p < 0.0005\)) than those of the matched group, \(27.41 \pm 7.59\) (mean \(\pm\) S.D.) \(\mu g/10^8\) WBC. The L.A.A. levels in different age groups are not significantly different from each other but in every age group the alcoholic group has a significantly lower vitamin C level when compared with the control group. When the diet histories were studied, it was clear that the main reason for low L.A.A. levels was inadequate intake of vitamin C.

Having established that alcoholics are generally deficient in vitamin C, an investigation was carried out to ascertain if this condition could directly affect the metabolism of ethanol. An experiment was designed to measure the induction of hepatic alcohol dehydrogenase by alcohol in sub-clinically scorbutic guinea pigs.

However, the criteria of sub-clinical scurvy (i.e. a state where there is a diminished level of ascorbic acid in the body without apparent manifestations of scurvy) had to be established at the outset. The leucocyte
ascorbic acid levels and the vitamin C levels in the liver, stomach and upper small intestine of guinea pigs were measured by the usual 2,4 dinitrophenyl hydrazine method. These levels show that guinea pigs are sub-clinically scorbutic after two weeks on a scorbutic diet.

Only male guinea pigs were used in the experiments. The levels of ADH were measured in the guinea pig livers after daily injections of 2 ml of 30% ethanol in saline for two days, one week, two weeks and three weeks. The guinea pigs in the scorbutic groups were always maintained on the scorbutic diet for two weeks.

The results show that ADH is maximally induced within two days and that there is a highly significant increase in ADH levels in sub-clinically scorbutic guinea pigs fed ethanol. The potentiation of ADH induction in sub-clinically scorbutic states could explain the increased tolerance of alcoholics for alcohol since sub-clinical scurvy was present in the majority of the cases of chronic alcoholism.

The level of NAD was measured in guinea pigs injected with 30% alcohol for two weeks and fed the scorbutic diet. The levels of NAD are higher in sub-clinically scorbutic animals fed ethanol than in normal animals fed ethanol.
CHAPTER V The levels of alcohol dehydrogenase in sub-clinically scorbutic guinea pigs

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INTRODUCTION.

ALCOHOL:

Alcoholic beverages have been used since the dawn of history. The oldest alcoholic drinks were fermented beverages of relatively low alcohol content, i.e. beer and wines. When the Arabs introduced the then recent science of distilling into Europe, in the Middle Ages, the Alchemists believed that alcohol was the long sought elixir of life. Alcohol was therefore held to be a remedy for practically all diseases. However, it is now recognised that the therapeutic value of alcohol is much more limited than its social value.

Ethanol plays at least five distinct roles in the body. The most widely appreciated is its effect on the central nervous system. In addition, ethanol is oxidised and utilised as a source of calories. Its metabolism in time yields acetaldehyde and acetate which exert pharmacologic and metabolic effects of their own. Ethanol may be hepatotoxic, leading to liver cell damage and, in some cases, eventually cirrhosis. Finally, alcohol acts as a drug similar to phenobarbital since it can induce microsomal drug detoxifying enzymes (1).

Alcohol is not a stimulant but rather is a primary and continuous depressant of the central nervous system. The apparent stimulation results from the unrestrained activity of various parts of the brain which have been freed from inhibition as a consequence of the depression of inhibitory control mechanisms.

Alcohol is absorbed from the stomach, small intestine and colon. Absorption of alcohol from the stomach is rapid
at first but it then decreases to a very slow rate although the gastric concentration of alcohol is still high. If the emptying of the stomach is delayed, the subsequent absorption of alcohol from the stomach will also be delayed (2). Absorption from the small intestine is rapid and complete and is independent of both the concentration of alcohol and the presence of food in the stomach or intestine. After absorption, alcohol is fairly uniformly distributed throughout all tissues and fluids of the body.

Of the alcohol entering the body, between 90 and 98% undergoes oxidation. The rest is excreted unchanged in the urine. The rate of oxidation of alcohol is a linear function of time and is only moderately increased by increasing the blood concentration. In the adult, the average rate of metabolism is 7g./hour. The initial oxidation of alcohol occurs in the liver and the important steps in the metabolism are outlined in Fig. 1. The acetate which is carried away from the liver by the blood is ultimately broken down into carbon dioxide and water (3). Some of the acetate may go into the formation of fat or cholesterol. Acetate can also be incorporated into amino acids via other intermediate steps. About 90% of the total amount of alcohol broken down in the body under normal conditions undergoes partial oxidation to acetate in the liver. The remaining 10% is broken down directly into carbon dioxide in different organs.

During the first two steps of the metabolism of 1gm. of alcohol - to acetaldehyde and acetyl CoA - approximately 2.6 kilocalories are liberated and are available to the body. Acetyl CoA or acetate enters the general
Fig. 1.

The main pathways are shown by heavy arrows.

![Diagram showing the metabolism of alcohol and acetaldehyde]

Ethanol → Acetaldehyde → Acetic acid

Ethanol → Acetaldehyde → Acetic acid

(1) Alcohol dehydrogenase (ADH)
(2) Acetaldehyde dehydrogenase
metabolic pool in the body and, provided that these substances are oxidised completely, another 4.5 kilocalories are available to the body. Thus the energy released by ethyl alcohol oxidation is approximately 7.1 kcal / g. Alcohol is a ready source of energy that is utilised more rapidly than most foods since it is quickly absorbed from the gastrointestinal tract and requires no preliminary digestion. Some alcoholic beverages also contain protein and carbohydrates but none contain any vitamins. Man is born with a very effective system which is able to break down alcohol and utilise the energy released thereby.

The enzyme alcohol dehydrogenase (ADH) was first isolated and crystallised from horse liver by Bonnichen and Wassen in 1948 and its properties were thoroughly studied by Theorell and his co-workers. In the presence of alcohol dehydrogenase ethanol reacting with nicotinamide adenine dinucleotide (NAD) is converted to acetaldehyde with the production of reduced nicotinamide adenine dinucleotide (NADH). Alcohol dehydrogenase is a zinc containing enzyme which is dependent upon free sulphydryl groups in the protein for its activity (4). It is present in the cytoplasm of liver cells and has a pH optimum of 10.8. Human liver ADH exhibits a broad substrate specificity e.g. 1,3 propane-diol and 2-methoxyethanol are oxidised by ADH. Alcohol dehydrogenase has a high affinity for NAD since alcohol is preferentially oxidised in the cell. Those substrates which are oxidised at a slower rate when alcohol is present are the ones that need NAD for their breakdown. All the substrates which are oxidised in the tricarboxylic acid
Several authors (5, 6) have reported an exceptionally active enzyme variant of human alcohol dehydrogenase which occurs in 20% of a Swiss and 4% of a London population. This form of the enzyme can be distinguished from the normal ADH because its pH optimum is 8.5. For many years the ADH system was thought to be the only one capable of oxidising ethanol. However, recently the oxidation of ethanol to acetaldehyde by the microsomal fraction of the liver has been reported (7). Several reaction mechanisms have been proposed. The system requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen and has a pH optimum of 7.4. Hoach et al. (8) stated that this microsomal system is mediated through hydrogen peroxide dependent systems, one of which is catalase. However, Lieber and de Carli (9) believe that the microsomal ethanol oxidising system is NADPH dependent but does not involve catalase. The importance of this system in the overall ethanol oxidation in the body is not clear. Certainly the existence of a microsomal ethanol oxidising system, especially its capacity to increase adaptively in activity after ethanol feeding, may explain various effects of ethanol, including proliferation of smooth endoplasmic reticulum, induction of other hepatic microsomal drug-detoxifying enzymes and the metabolic tolerance to ethanol which develops in alcoholics.

In man there are four common hepatic disorders that are thought to be related to the ingestion of ethanol or ethanol-containing beverages because of the fact that these
disorders are more common in the drinker than in the non-drinker. These disorders are fatty infiltration of the liver, acute alcoholic hepatitis, Laennec's cirrhosis and haemachromatosis (10).

The interactions of alcohol and living material have been well studied, well analysed and should be well understood. That the latter is not completely so does not detract in any way from the benefits accessible to mankind from the prudent use of alcohol.
Scurvy was the first deficiency disease to be recognised as such. As early as 1720, Kramer observed that medicines gave no relief to this condition, but "if you can get green vegetables, if you can prepare a sufficient quantity of fresh antiscorbutic juices, if you have oranges, lemons, citrons or their pulp and juice preserved with whey in cask so that you can make a lemonade or rather give to the quantity of 3 or 5 ounces of their juice in whey, you will, without other assistance, cure this dreadful evil."

In his exhaustive treatise on scurvy, Lind (1757) showed experimentally, in human subjects, that oranges and lemons incorporated in the diet could prevent the onset of the disease. In 1804 it became compulsory in Britain to issue a daily ration of lemon or lime juice to all British sailors. This led to the almost complete eradication of scurvy in the British navy. Holst and Frölich (1907) showed that experimental scurvy could be produced in guinea pigs and this provided a bioassay for the antiscorbutic vitamin. In 1921 Zilva discovered that vitamin C could be easily destroyed by oxidation and could best be protected by reducing agents. In 1928 Szent-Györgyi isolated a powerful reducing agent in crystalline form from the adrenal cortex. He believed it to be a hexuronic acid derivative having the empirical formula $C_6H_6O_6$. He also isolated this compound from cabbage and orange juice and recognised this crystalline material as probably being identical to the reducing agent reported by Zilva in vitamin C concentrates. King and Waugh (1932) finally succeeded in isolating a crystalline compound from lemon juice concentrates,
identified it as hexuronic acid and demonstrated its antiscorbutic properties. A few weeks later, Svirbely and Szent-Györgyi (1932) announced that hexuronic acid obtained from adrenal glands, cabbage and oranges was also highly antiscorbutic. Shortly afterwards, the exact chemical constitution of the antiscorbutic hexuronic acid was established independently by workers in several laboratories. It was also synthesised chemically. Because of its physiological role in the prevention of scurvy, the compound was called ascorbic acid.

Ascorbic acid is reversibly oxidisable in the body producing dehydroascorbic acid, which possesses full vitamin activity. The physiological functions of the vitamin are probably related to the oxidation-reduction system. The structural formulae involved are:

\[
\begin{align*}
\text{Ascorbic acid} & : & \begin{array}{c}
\text{O} \\
\text{OH} \\
\text{OH} \\
\text{H} \\
\text{OH} \\
\text{CH}_2\text{OH}
\end{array} & \leftrightarrow & \begin{array}{c}
\text{O} \\
\text{OH} \\
\text{OH} \\
\text{H} \\
\text{OH} \\
\text{CH}_2\text{OH}
\end{array}
\end{align*}
\]

Dehydroascorbic acid
Ascorbic acid is an optically active compound. Hughes and Hurley (11) have shown that when guinea pigs on a low, controlled intake of ascorbic acid (0.3 mgs / 100 gms body weight) were given a daily oral dose of L-ascorbic acid or of its isomer D-isoascorbic acid (both 1.5 mgs / 100 gms body weight), the ascorbic acid was deposited in the tissues while D-isoascorbic acid was not. They concluded that in guinea pigs ascorbic acid is absorbed from the gastrointestinal tract much more readily than isoascorbic acid and ascorbic acid is either more readily abstracted from the blood or is more readily retained by the tissues, or both, than is isoascorbic acid. Hughes and Jones (12) found that the presence of isoascorbic acid in the drinking water overcomes its low absorption efficiency and results in substantial tissue levels. The appearance of the normally accepted symptoms of scurvy may, in this way, be suppressed by isoascorbic acid although the growth rate does not equal that of ascorbic acid sufficient guinea pigs. Although isoascorbic acid and other compounds closely related chemically to ascorbic acid do possess some antiscorbutic activity, none compares in potency with the natural vitamin.

In the strict sense of the word, vitamin C may be said to posses few pharmacological actions. Prolonged high dosage of ascorbic acid does not have any qualitative or quantitative effect on the body (13, 14). In the scorbutic individual however, administration of the vitamin leads to a rapid alleviation of the symptoms.

Ascorbic acid and dehydroascorbic acid form a redox system with semidehydroascorbic acid as a highly
reactive intermediate (15). Almost all metabolic processes
the disturbance of which give rise to scurvy, involve
reactions in which ascorbic acid is oxidised. The formation
and maintenance of collagen is dependent on a normal
ascorbic acid level (16). Similarly dependent on ascorbic
acid is the hydroxylation of the side chain of dopamine
which results in the formation of noradrenaline.

Some authors (17, 18) have suggested that ascorbic
acid is involved in the hydroxylations occurring in steroid
synthesis in the adrenals because the secretion of these
steroids is reduced in ascorbic acid deficient patients.
Others (19) have proposed that scurvy appears to involve
such severe stress which results in an increase in adreno­
cortical secretion, that the synthesis rate of cortisol
and corticosterone is incapable of matching the rate of
release of these steroids.

Ascorbic acid is also concerned in tyrosine metabolism,
as a reducing agent in the conversion of folic to tetra­
hydrofolic acid and in the incorporation of iron into
ferritin (20). These latter properties may account for
the anaemia of scurvy which has been reported by various
authors (21, 22). Ascorbic acid also serves some definite
metabolic function in platelet aggregation (23).

Ascorbic acid is involved in carbohydrate metabolism,
as evidenced by the fact that scorbutic animals exhibit
hyperglycaemia and low hepatic glycogen content and are
resistant to insulin.

In contrast to the reactions dependent on ascorbic
acid, the hydroxylation of tryptophan to 5-hydroxytryptophan,
the precursor of serotonin, is mediated by dehydroascorbic acid which is thereby reduced to ascorbic acid (24). The regeneration of ascorbic acid from dehydroascorbic acid in the tissues plays an important metabolic role. Ascorbic acid is synthesised by higher plants and all animals except primates and guinea pigs. Animals which do not form ascorbic acid lack the enzyme L-gulonolactone oxidase, required to convert L-gulonolactone to L-ascorbic acid. Ascorbic acid is absorbed in the gastrointestinal tract in the same way as glucose and other carbohydrates (25). Equilibrium between ascorbic acid absorbed from the diet and that present in the tissues is attained in less than four hours. The normal level of ascorbic acid in leucocytes is 20 - 50 µg. / 10^8 W.B.C. (26). The leucocyte ascorbic acid levels depend on the degree of tissue saturation and on dietary intake. With an ascorbic acid concentration of 20 mgs / kg. body weight, the daily turnover is about 1 mg. / kg., corresponding to a half life of 16 days (27). In man, the main route of excretion is through the urine with trace amounts expelled in the faeces and respiratory carbon dioxide. In urine, ascorbic acid appears mainly unchanged but part is hydrolysed to diketogulonic acid and appears finally as oxalic acid (25). Other metabolites are probably derivatives of L-threose or L-threonic acid (28,29).

The British Medical Association Committee on Nutrition (1950) recommend a daily intake of vitamin C of 30 mgs per person per 24 hours to provide a good margin of safety for the prevention of scurvy. It has been suggested that up to 10% of the households in Britain and
an even greater proportion of individuals may have a vitamin C intake which is permanently below this prescribed level (30).

The most striking manifestation of ascorbic acid deficiency is a marked tendency to bleeding with the appearance of extensive patches of haemorrhage under the skin and in the gums, muscles, fatty tissues and internal organs. Hansky (31) found that 15 of a sample of 31 patients in whom the cause of bleeding remained undetermined, had a low vitamin C intake. Other secondary features of ascorbic acid deficiency are impairment of connective tissue formation, leading to poor wound healing, roughening of the skin (hyperkeratosis) and anaemia.

The present work reports nutritional studies, with particular reference to the role of vitamin C, on alcoholics and on guinea pigs which had been fed ethanol. An investigation of the effect of home-brewed material (lager) on the gastric mucosa of healthy guinea pigs and of man was also undertaken and the findings are presented in a succeeding Chapter.
CHAPTER II
Gastric erosions caused by home-brewed lager.

The fermentation of sugars by yeast to produce ethanol has been known since antiquity. Ethanol is the sole product of yeast fermentation under anaerobic conditions. Other fermentative products i.e. acetaldehyde and acetic acid, will be produced if the yeast is infected by certain other bacteria.

Seven years ago, restrictions governing the home-brewing of beer were removed and, since then, home-brewing has become ever more popular. Many types of brewing kits have become available. Each includes fresh hops, yeast, malt or malt extract and salts, which are required for fermentation. The main attractions of these kits are the ease of operation and their low cost.

These studies were prompted by the admission of a patient with melaena, who had recently ingested large amounts of home-brewed lager.

CASE REPORT.

A 22-year old electrician was admitted to Stobhill General Hospital in October 1970 with abdominal pain, nausea and vomiting and melaena. There was no history of previous dyspeptic symptoms nor of aspirin ingestion. A few weeks previously, he had brewed lager at home, using a kit which is widely sold. He made about five gallons of this mixture and had drunk most of it himself over a period of three weeks.

On examination, he was not shocked; blood pressure 120 / 60; pulse 105 per minute. Apart from generalised pallor, there was no clinical abnormality. His haemoglobin on admission was 11.8 g.% and his P.C.V. was 35%. Over the
the subsequent few days, his haemoglobin fell to 9.8 g% and his P.C.V. to 33%. A barium meal showed no abnormality of the oesophagus, stomach or duodenum. Fibroscopy five days after admission revealed that the gastric rugal pattern was normal, the mucosa was not injected but one superficial erosion was seen in the anterior wall of the pyloric canal. The patient had no recurrence of his symptoms.

The clinical diagnosis was one of erosive gastritis. It was considered that this could have been due to the ingestion of the home-brewed lager and studies were carried out to investigate this possibility.

**MATERIALS AND METHODS.**

Five gallons of beer and five gallons of lager were brewed in the laboratory from a kit similar to that used by the patient (Fig. 2.1). A sample of laboratory-brewed beer and lager, the patient's lager and commercial pale ale and lager were analysed by gas-liquid chromatography in a Pye 104 Chromatograph. This work was conducted in the Department of Forensic Medicine in the University of Glasgow, by Dr. Hamilton Smith. Resolution of the components was achieved on a column 5 ft. long packed with chromosorb 101. The operating temperature was 170°C and the rate of nitrogen gas flow was 60 ml/minute. The internal standard was n-propanol, at a concentration of 8 mg/100 ml. water. Samples of the patient's lager, laboratory-brewed beer and lager and fresh yeast straight from a lager kit were analysed bacteriologically by Mr. Peter Owen of the Department of Microbiology, University of Glasgow.
Figure 2.1. Photograph of the kits used to brew lager and beer.
ANIMAL STUDIES.

Thirty-six male guinea pigs - weight range 330 - 580 gms - were fed on the standard guinea pig diet 18 (Table 2.1) based on the recommendations laid down by the U.F.A.W. handbook (1957) (32). The guinea pigs were divided into three groups each group comprising twelve animals (Table 2.2). One group was given measured aliquots of tap water as their sole drinking fluid, another group was given measured aliquots of laboratory-brewed lager and a third group was given measured aliquots of laboratory-brewed beer. In addition, each animal was given a small amount of the appropriate liquid into their stomach. On days 1 and 2 they were each given 2 mls by intubation tube; on day 3 they were given 4 mls and on day 4 they were given 5 ml. The animals did not fast before force-feeding but were lightly anaesthetised with ether. The animals were killed on day 5.

In a second experiment, thirty-six male guinea pigs - weight range 250-680 gms - were divided into three groups. The experiment was repeated exactly as before but the animals were not force-fed (Table 2.2). The guinea pigs were killed by ether anaesthesia and the stomachs were dissected. The gastric mucosa were examined carefully by the naked eye for the presence of bleeding points (defined as the obvious continued oozing of blood from a localised point on the mucosa after careful swabbing). The gastric mucosa of each animal was also tested for the presence of blood by the orthotolidine method (33). The mucosa was tested by placing drops of the reagent on the mucosa of the whole stomach after removal from the body. In each case the number of seconds
### TABLE 2.1 COMPOSITION OF NORMAL DIET FOR GUINEA PIGS.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass meal</td>
<td>30</td>
</tr>
<tr>
<td>Bran</td>
<td>15</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>15</td>
</tr>
<tr>
<td>Linseed cake</td>
<td>10</td>
</tr>
<tr>
<td>Barley</td>
<td>20</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>8</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>1</td>
</tr>
</tbody>
</table>

### TABLE 2.2 GUINEA PIG EXPERIMENTAL GROUPS.
(12 animals in each group)

<table>
<thead>
<tr>
<th>Guinea Pig Diets</th>
<th>Food</th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Diet 18 + Cabbage daily</td>
<td>Water</td>
</tr>
<tr>
<td>Group B</td>
<td>Diet 18 + Cabbage daily</td>
<td>Lager - Hosp. brew</td>
</tr>
<tr>
<td>Group C</td>
<td>Diet 18 + Cabbage daily</td>
<td>Beer - Hosp. brew</td>
</tr>
</tbody>
</table>
taken for the blue colour to develop was estimated by stopwatch.

One stomach from each group of animals in the first experiment, i.e. with intubation, was examined histopathologically. The stomach was not sectioned serially but only random sections were examined. The slides were stained with Haematoxylin and Eosin.

RESULTS.

The results of Gas-Liquid Chromatography analysis are shown in Fig. 2.2. The peak heights are not proportional to the concentrations of the components of the mixture. The contaminants were present only in trace amounts and therefore their percentage composition in the lager could not be determined. The ethanol content of all the lager samples was approximately the same. Both the patient's lager and the laboratory-brewed lager contained traces of acetaldehyde and acetic acid. The patient's lager had more of these contaminants than had the laboratory brew. The laboratory brew also contained traces of ethyl acetate which could have been formed by the mass action esterification of acetic acid. In contrast, the laboratory-brewed beer was free from any contaminating substance.

These contaminants are formed by different fermentative pathways (Fig. 2.3). The main fermentative pathway is that shown across the top of Figure 3, and occurs when yeast is growing and multiplying under anaerobic conditions. If there are any extraneous bacteria present, ethanol will be metabolised by different routes. These pathways require enzymes which are not present in yeast.
Fig. 2.2  G.L.C. ANALYSIS OF BEERS AND LAGERS.

<table>
<thead>
<tr>
<th>Component</th>
<th>Hospital Lager</th>
<th>Patient's Lager</th>
<th>Tennent's Lager</th>
<th>Hospital Beer</th>
<th>McEwan's Pale Ale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyl Alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol 3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol 3.4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol 3.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol 3.6%</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ethanol 2.6%</td>
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</tr>
</tbody>
</table>
Acetic acid can be produced via acetyl CoA or directly from acetaldehyde. Direct conversion of acetaldehyde to acetic acid occurs only in alkaline medium. The laboratory-brewed beer was free from bacterial contaminants, but both the patient's lager and the laboratory-brewed lager had an infection of Acetobacter melanogenus which is known to produce acetic acid from ethanol. The dry lager yeast also had this infection.

On histopathological examination, the sections of the stomachs from the beer and lager drinking animals showed much greater congestion than the stomachs of the water-drinking animals (Figs 2.4, 2.5, 2.6). The congestion was superficial and there was no cellular infection. The irritating effect
of the lager is clearly seen in Fig. 2.7. This mucosal reddening was present in most of the beer and lager drinking animals. There was no visual difference in the extent of mucosal reddening between the beer and lager drinking animals. Obvious bleeding points were found significantly more often in the alcohol drinking animals which were also receiving aliquots by intubation tube. When the animals were not force-fed, there was no significant difference in the number of visible bleeding points (Table 2.3).

The results of the orthotolidine tests on the gastric mucosa are shown in Table 2.4. The differences between these patterns were analysed by the Mann-Whitney test (Table 2.5). The contaminated lager was significantly more irritant than the beer \((p<0.02)\) in intubated animals. In the second experiment, when the animals were drinking the fluids but were not being force-fed, there was a significant difference \((p<0.05)\) between the lager-drinking group and the water-drinking group, but not between the beer and water drinking groups.
Fig. 2.4  Section of stomach of a water-drinking animal. The gastric lumen appears at the top of the photograph. The stomach was stained with H and E, thus blood vessels appear orange in colour.
Fig. 2.5 Section of stomach from a lager-drinking animal (hospital-brewed). The gastric lumen appears at the top right hand side of the photograph. Note the large increase in the number of blood vessels as compared with Fig. 2.4.

Fig. 2.6 Section of the stomach from a beer-drinking animal (hospital-brewed), the lumen appearing at the right hand side of the figure. This stomach is also congested.
Fig. 2.7 Guinea pig stomachs.

The pale mucosa on the right came from a guinea pig which drank only water. The inflamed mucosa on the left came from a guinea pig which drank hospital-brewed lager and was given additional aliquots of this lager by gastric intubation.
**TABLE 2.3**

Number of guinea pigs with visible bleeding points on gastric mucosa. All animals fed on Diet 18 + cabbage.

<table>
<thead>
<tr>
<th>Drinking fluid</th>
<th>12 animals/group with intubation</th>
<th>12 animals/group without intubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Lager</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Water</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

**TABLE 2.4**

Comparison of bleeding of gastric mucosa by the orthotolidine test on guinea pigs' stomachs. All animals fed Diet 18 + cabbage. Each cross represents one guinea pig.

<table>
<thead>
<tr>
<th></th>
<th>With intubation</th>
<th>Without intubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beer     Lager  Water</td>
<td>Beer    Lager   Water</td>
</tr>
<tr>
<td>Negative</td>
<td>++       ++++    ++++</td>
<td>+++     ++      ++++</td>
</tr>
<tr>
<td>Positive</td>
<td>++++     +++     ++++</td>
<td>+++     +++      ++++</td>
</tr>
<tr>
<td>30 secs</td>
<td>++       ++      ++</td>
<td>++      +++      ++</td>
</tr>
<tr>
<td>25 secs</td>
<td>+        +++      ++</td>
<td>++      +++      +</td>
</tr>
<tr>
<td>20 secs</td>
<td>+        +++      +</td>
<td>+       +++      +</td>
</tr>
<tr>
<td>10 secs</td>
<td>+        +++      +</td>
<td>+       +++      +</td>
</tr>
<tr>
<td>0 secs</td>
<td>+        +++      +</td>
<td>+       +++      +</td>
</tr>
</tbody>
</table>
### Results of Mann-Whitney tests.

(from orthotolidine tests on guinea pig stomachs - Table 2.4)

<table>
<thead>
<tr>
<th></th>
<th>U value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>With intubation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lager and water</td>
<td>16</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Beer and water</td>
<td>35</td>
<td>0.1</td>
</tr>
<tr>
<td>Lager and beer</td>
<td>36</td>
<td>N.S</td>
</tr>
<tr>
<td><strong>Without intubation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lager and water</td>
<td>32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Beer and water</td>
<td>50</td>
<td>N.S</td>
</tr>
<tr>
<td>Lager and beer</td>
<td>55.5</td>
<td>N.S</td>
</tr>
</tbody>
</table>

(N.S : Not Significant)
DISCUSSION.

The results suggest that the laboratory-brewed lager is more irritant to the gastric mucosa than the laboratory-brewed beer. This difference could be attributed to the harmful contaminants, acetaldehyde and acetic acid, present in the lager. These contaminants were present only in trace amounts, but acetaldehyde is extremely toxic.

Augur (34) showed that 9% (w/v) ethanol or 100 mM acetic acid can cause congestion in the stomachs of dogs with Heidenhaem pouches. In addition, it is known that the long continued use of strong alcoholic beverages in man may result in chronic gastritis in approximately one out of three individuals (35). These figures are valid as long as the alcoholic beverages are not contaminated with other harmful substances. The laboratory-brewed beer was not contaminated and did not produce any significant irritation of the gastric mucosa of guinea pigs, over a period of four days, in contrast to the contaminated beverages.

Home-brewing of beer, lager and also wine has become very popular since the introduction of these kits. The main advantage is the low cost, but it now appears that home-brewing is not without danger, particularly in the bacterial contamination of the yeast, which occurred in all three samples of the lager yeast. If the yeast is contaminated, the liquid will taste and smell sour and this should be a deterrent to the consumer. These findings suggest that the consumer requires to be protected either by Public Health legislation or by greater quality control measures on the part of the producer.
CHAPTER III
A clinical and nutritional study of alcoholics.

According to the World Health Organisation, "alcoholics are those excessive drinkers whose dependence upon alcohol has attained such a degree that it interferes with their bodily and mental health, their interpersonal relationships and their smooth social and economic functioning or who show early signs of such developments". This definition could be applied to all patients interviewed in this study.

It has been apparent for many years that alcoholics, in addition to consuming ethanol, do not as a rule consume a diet that bears any close resemblance to that of non-alcoholics (10). It is also true that just as ethanol consumption will modify nutritional requirements, diet modifies the effect of ethanol.

Alcoholic patients admitted to the Medical Unit at Glasgow Western Infirmary have often been noted to have a low level of Leucocyte Ascorbic Acid (LAA). The cause of this may be dietary in many cases but another possible explanation for this is the accelerated oxidative catabolism of ascorbic acid as occurs in the siderotic Bantu (36). It was decided to test these theories by measuring leucocyte ascorbic acid and comparing this level with the dietary intake of vitamin C obtained from an analysis of the average daily food and drink intake of the patients.

Both alcohol and vitamin C have an effect on iron metabolism. Alcohol stimulates the absorption of iron from the small intestine but it has a detrimental effect
on haemopoiesis (37). In ascorbic acid deficiency, iron absorption is greater than normal in response to an increased erythropoietic activity, but the metabolism of storage iron is abnormal (38). Therefore either prolonged alcohol ingestion or vitamin C deficiency has frequently been associated with anaemia.

In order to assess the effect of ethanol at a known level of leucocyte ascorbic acid, the serum iron, the total iron binding capacity and the erythrocyte porphyrins both erythrocyte protoporphyrin and erythrocyte coproporphyrin levels, were measured along with the leucocyte ascorbic acid level in alcoholic patients. The dietary intake of iron was also assessed from an analysis of the diet histories.

**Materials and Methods.**

A survey of fifty alcoholics, ages ranging from 24 to 70 years, was carried out. The group included nine female patients, age range 34 - 50 years. Most of the patients were seen in hospital but some were interviewed at a voluntary care centre for alcoholics. None of the patients had overt liver disease. The blood sample for LAA, packed cell volume (P.C.V.), serum iron, total iron binding capacity and erythrocyte porphyrins estimation was withdrawn as soon as possible after admission to hospital. Haemoglobin levels were estimated in 40 of the patients. None of the patients were intoxicated at the time the blood sample was taken. A complete dietary history was recorded from each patient. The alcoholic group was matched according to age (within five years)
and sex with a normal group of patients.

Leucocyte ascorbic acid was estimated by the 2,4-dinitrophenol method (39), which is described overleaf and the average daily intake of vitamin C and iron was calculated from the diet history. Serum iron and total iron binding capacity were measured by the method of Ramsay (40) and erythrocyte porphyrins, both erythrocyte protoporphyrin and erythrocyte coproporphyrin, were measured by the method of Rimington (41).

**Method for the measurement of leucocyte ascorbic acid.**

All estimations of leucocyte ascorbic acid must be started within four hours of withdrawal of the blood from the patient. Three to four millilitres of fresh whole blood are added to a bottle containing 12.5 ml of a mixture of 200 ml of 0.9% saline / 50 ml of 6% dextran (M_w = 125,000) / 2 ml of 10% sequestrene (42), mixed thoroughly and allowed to stand for one half hour at room temperature, during which time most of the erythrocytes have separated. 10 ml of the supernatant fluid are taken for the estimation and the remainder is used for a leucocyte count. The 10 ml of supernatant fluid are spun at 3,000g for 15 minutes to bring down the white cells and platelets. The supernatant is discarded and the cellular deposits are ground with 1.3 ml of 5% trichloroacetic acid. At this stage, the estimation can be left overnight at 2°C.

The specimen is then centrifuged at 3,000g for ten minutes to remove cell debris and 1 ml of the resulting supernatant fluid is incubated with 0.3 ml of 100 vols
of 2.2 g of 2,4 dinitrophenyl hydrazine in 10 N sulphuric acid / 5 vols of 5% thiourea / 5 vols of 0.6% copper sulphate reagent (42) at 37°C for four hours. After cooling in ice-water, 1.5 ml of 65% sulphuric acid is added and the absorbance is read against a reagent blank at 520 nm in a spectrophotometer. A calibration graph can be prepared by using concentrations of 0.0, 0.5, 1.0, 2.5, 3, 4 and 5 µg ascorbic acid / ml in 5% trichloroacetic acid, these being treated identically to the test solution. The test readings can then be read off the straight line graph thus obtained. The ascorbic acid content of the leucocytes is expressed as µg of ascorbic acid / 10^8 leucocytes and is calculated as follows :

\[
\text{Graph reading} \times 1.3 \times 100
\]
\[
\text{Leucocyte count in hundreds /c.mm.}
\]
\[
= \frac{\text{µg ascorbic acid} \times 10^8}{\text{leucocytes}}.
\]

**RESULTS.**

The range of leucocyte ascorbic acid levels in the alcoholic group is 0-50 µg/10^8 WBC (Fig. 3.1) with a mean of 18.18 ± 11.01 (mean ± S.D.) µg/10^8 WBC. The range in the control group is 11.46 µg/10^8 WBC (Fig. 3.2) with a mean of 27.41 ± 7.59 (mean ± S.D.) µg/10^8 WBC. Thus the vitamin C levels of the alcoholics are significantly lower (p<0.0005) than those of the matched group (Fig. 3.3).

It is seen in Table 3.1 that this vitamin C deficiency occurs in every age group in the alcoholic sample, and in both sexes. The vitamin C levels in different age groups are not significantly different from each other, but in every age group, the alcoholic patients
Fig. 3.1

No. of Patients.

(Fig. 3.1) Normal range

Leucocyte ascorbic acid (\(\mu g/10^8\) WBC)

---

Fig. 3.2

Leucocyte ascorbic acid levels in compared normal patients (Every rectangle denotes one patient).

No. of Patients.

(Fig. 3.2) Leucocyte ascorbic acid levels in compared

Leucocyte ascorbic acid (\(\mu g/10^8\) WBC)
Leucocyte ascorbic acid levels in normal and alcoholic humans.

L:A:A. (µg/10^8 WBC)

Table 3.1: Leucocyte ascorbic acid levels in normal and alcoholic humans in different age groups.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Alcoholics (µg/10^8 WBC; mean ± S.D.)</th>
<th>Controls (µg/10^8 WBC; mean ± S.D.)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 45 (25)</td>
<td>17.2 ± 11.9</td>
<td>25.1 ± 7.7</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Under 45 (25)</td>
<td>19.4 ± 10.0</td>
<td>27.7 ± 7.9</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Under 30 (9)</td>
<td>16.8 ± 10.6</td>
<td>27.7 ± 9.4</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>Females (9)</td>
<td>21.3 ± 8.2</td>
<td>26.2 ± 1.8</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Fig. 3. L.A. and diet.

L.A.A.

\( \mu g / 10^8 \text{ WBC} \)

- Represents one alcoholic patient.
- Represents one control patient.

L.A.A. of 20 \( \mu g / 10^8 \text{ WBC} \) is the lower limit of normal range.

Vitamin C intake of 30 mg/24 hours is recommended by the B.M.A.
have a significantly lower L.A.A. level than the control group.

The comparison of vitamin C intake with leucocyte ascorbic acid in both the alcoholic and control patients is shown in Fig. 3.4. The British Medical Association recommend an intake of vitamin C of at least 30 mg per person per day to maintain adequate health and prevent any signs of scurvy. Half of the alcoholic patients (25 out of 50) have a low leucocyte ascorbic acid level because of insufficient intake. Only two subjects in the control group have a low leucocyte ascorbic acid because their intake is less than 30 mg per day. Five alcoholic patients and seven control patients have an adequate intake of vitamin C but their leucocyte ascorbic acid levels are below 20 μg/10^8 WBC. These patients may break down vitamin C faster than normal or may not absorb it adequately. Only eleven alcoholic patients compared with 29 out of 50 control subjects have an adequate vitamin C intake and a leucocyte ascorbic acid level above 20 μg/10^8 WBC.

The results of haemoglobin and packed cell volume measurements and white blood cell counts in normal and alcoholic patients are summarised in Table 3.2.

The normal levels of erythrocyte coproporphyrin (E.C.P.) for both males and females are 1.78 ± 1.3 μg/100 ml red blood cells (R.B.C.). The accepted range is 0 - 4.0 μg/100 ml of red blood cells (43). The erythrocyte coproporphyrin levels found in forty alcoholics were 1.8 ± 1.6 (mean ± S.D.) μg/100 ml of R.B.C. The range was 0 - 7.6 μg/100 ml R.B.C. The erythrocyte coproporphyrin
**TABLE 3.2**

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Normal level (mean ± S.D.)</th>
<th>Alcoholic level (mean ± S.D.)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemoglobin</strong></td>
<td>Male (35)</td>
<td>100% (16g/100 ml blood)</td>
<td>100% ± 9.9%</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Haemoglobin</strong></td>
<td>Female (5)</td>
<td>100% (14g/100 ml blood)</td>
<td>86.4% ± 15.9%</td>
<td>N.S.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 28.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Packed cell volume</strong></td>
<td>Male (39)</td>
<td>46.2% ± 3%</td>
<td>45.6% ± 4.6%</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>Packed cell volume</strong></td>
<td>Female (8)</td>
<td>40.6% ± 4.8%</td>
<td>37.3% ± 9.2%</td>
<td>N.S.</td>
</tr>
<tr>
<td><strong>White blood cell counts</strong></td>
<td>Male and Female (28)</td>
<td>7,500 ± 2,500 WBC/c.mm.</td>
<td>7,550 ± 2,600 WBC/c.mm.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
level was above 4 μg/100 ml R.B.C. in only four alcoholic patients. Therefore the E.C.P. levels in normal and alcoholic patients are not significantly different.

The normal values for erythrocyte protoporphyrin (E.P.P.) for males and females are 24.03 ± 12.8 (mean ± S.D.) μg/100 ml R.B.C. The values found in forty alcoholic patients were 43.3 ± 34.65 (mean ± S.D.) μg/100 ml R.B.C. with a range of 11-161 μg/100 ml R.B.C. In the alcoholic group, 14 out of 36 patients had an E.P.P. greater than 35 μg/100 ml. These results must be considered with the serum iron and total iron binding capacity (T.I.B.C.) results.

Serum iron levels were measured in 34 male alcoholics. The mean of these levels was 120 ± 75.7 (mean ± S.D.) μg% and the range of 20-358 μg% is significantly different from the normal range for men which is 90-140 μg%. Twelve patients had a serum iron level lower than 90 μg%. This could arise from insufficient intake of iron. When each diet history was analysed, it was found that every alcoholic patient had an intake less than the recommended intake of 15 mg of iron per day. Eight patients (approximately 25%) had a serum iron level greater than 140 μg%. Of these, five patients had a serum iron level in excess of 200 μg%.

The range of total iron binding capacities in alcoholic patients was 180-558 μg% compared with a range of 250-400 μg% in normal patients.

The serum iron results and the T.I.B.C. results were used to evaluate the percentage saturation of iron
Fig. 3.5 The % saturation of iron compared with the erythrocyte protoporphyrin levels in a group of 36 alcoholic patients. (each cross denotes one alcoholic patient)

E.P.P. = 35 µg% is the upper limit of normal range.

% saturation = 16% is the lowest acceptable value.
Fig. 3.6 The correlation of % saturation of iron with erythrocyte protoporphyrin levels in 36 alcoholic patients.

% saturation of iron.

36 samples, p < 0.05.
in the blood of the alcoholic patients. The % saturation of iron was compared with the E.P.P. levels (Fig. 3.5) to establish the incidence of anaemia in this alcoholic group. The figure shows five cases of iron deficiency anaemia, i.e. a saturation of the total iron binding capacity of less than 16% and an E.P.P. level higher than 35 μg/100 ml R.B.C. The normal saturation of iron is about 33%. Over 50% of the patients show no signs of abnormal iron metabolism since their iron saturation is greater than 16% and the E.P.P. values are between 0-35 μg/100 ml R.B.C. There is a statistically significant correlation (p < 0.05) between the % saturation of iron and the E.P.P. levels in 36 estimations (Fig. 3.6). The asymptotic curve has the equation y = ax^b where : a = 70.13 and b = -0.25, and the coefficient of correlation is -0.28.

DISCUSSION.

The results show that, as a group, alcoholics are sub-clinically scorbutic and that this is caused by insufficient dietary intake of vitamin C. There is no evidence from these results that the catabolism of vitamin C is increased in alcoholics nor is there any reason to suggest that alcoholics require more than 30 mg of vitamin C per 24 hours.

The aetiology of the anaemia caused by prolonged alcoholic ingestion has been variously ascribed to gastrointestinal blood loss, dietary deficiency of iron and folic acid, increased haemolysis and depressed haemopoiesis.
The subject has been reviewed by Kimber et al (44). Similarly, approximately 90% of patients with scurvy have anaemia with a haemoglobin level of below 12 g/100 ml of blood (45). The cause of this is not clear but the anaemia of scurvy has been attributed to the same factors as those described above.

It has been shown that alcoholics are not folate deficient (46), but in this survey, all alcoholics had an inadequate intake of iron. The incidence of anaemia in these alcoholics is lower than expected from the dietary results, since only five alcoholic patients had iron deficiency anaemia.

The enzyme ferrochelatase catalyses the insertion of iron into protoporphyrin to produce haem. Therefore the level of protoporphyrin in the blood is elevated if there is a deficiency of iron or a depression of the activity of ferrochelatase. It is known that alcohol depresses ferrochelatase activity (43). In this study there are five patients with raised E.P.P. levels but their percentage saturation of iron is greater than 16%. This anaemia is due to depressed haemopoeisis.

Five patients have a serum iron level of over 200 μg% which is indicative of active hepatocellular damage although none of the alcoholics had a clinical diagnosis of hepatocellular failure.

The haemoglobin and the P.C.V. levels show no difference between the normal and the alcoholic patient. 55% of these alcoholic patients show no sign of anaemia on the basis of these haemoglobin levels. Therefore,
in the absence of liver disease, the incidence of anaemia produced by prolonged alcohol ingestion is much lower than expected.
The criteria of sub-clinical scurvy in guinea pigs.

Sub-clinically scorbutic guinea pigs were formerly defined as guinea pigs on a low ascorbic acid diet without any signs of frank scurvy. Clinical signs of scurvy in guinea pigs consist principally of emaciation, atrophy of mucosal surfaces and petechial haemorrhages on the serous surfaces (32). This definition of sub-clinical scurvy is very vague and thus it was decided to establish biochemical criteria for the condition by measuring vitamin C levels in leucocytes and in other tissues (liver, stomach and upper small intestine) of guinea pigs fed on a normal or a scorbutic diet. Previous attempts to measure ascorbic acid levels in guinea pig leucocytes were unsuccessful because of the difficulty in separating the buffy layer.

In this chapter a method is described for the separation of guinea pig blood buffy layer, and this technique was subsequently used for measurement of leucocyte ascorbic acid in guinea pigs.

Materials and Methods.

Six male guinea pigs, weight range 150-250 g. were caged individually in cages with a covering of peat and wood straw. They were fed diet 18 (Table 4.1) for two weeks. Their sole drinking fluid was tap water and they were not fed cabbage. After two weeks the animals were killed by ether anaesthesia. Between three and four millilitres of blood were withdrawn by cardiac puncture just before death and this was used for L.A.A. estimation as described overleaf. The vitamin C content of the liver, stomach
### TABLE 4.1 Normal diet given to guinea pigs.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass meal</td>
<td>30</td>
<td>Barley</td>
<td>20</td>
</tr>
<tr>
<td>Bran</td>
<td>15</td>
<td>Meat and bone meal</td>
<td>8</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>15</td>
<td>Salt</td>
<td>1</td>
</tr>
<tr>
<td>Linseed cake</td>
<td>10</td>
<td>Ground limestone</td>
<td>1</td>
</tr>
</tbody>
</table>

This diet has 164 mg/kg of ascorbic acid. According to the Handbook of Biological Data, guinea pigs, both male and female, are estimated to require 100-200 mg of ascorbic acid /kg of diet for normal growth.

### TABLE 4.2 Ascorbic acid deficient diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skimmed milk powder</td>
<td>30</td>
<td>Vegetable oil</td>
<td>8</td>
</tr>
<tr>
<td>Ground rolled oats</td>
<td>39</td>
<td>Cod liver oil</td>
<td>2</td>
</tr>
<tr>
<td>Fine wheat bran</td>
<td>20</td>
<td>Salt</td>
<td>1</td>
</tr>
</tbody>
</table>

This diet has 81 mg of ascorbic /kg of diet.
and upper small intestine was measured in each guinea pig by the method described overleaf.

Eighteen male guinea pigs, weight range 150-250 g. were fed a scorbutic diet (Table 4.2) and given tap water ad libitum. Six guinea pigs were killed by ether anaesthesia at the end of one week, another six were killed after two weeks and the remaining six animals were killed at the end of three weeks. The leucocyte ascorbic acid and the vitamin C level in the liver, stomach and upper small intestine was measured in each animal.

The percentage water content of each guinea pig tissue was measured by leaving a weighed sample of the tissue in an oven at 110°C for several days until the dry weight was constant.

The optimum molecular weight dextran for the separation of guinea pig buffy layer.

Vitamin C is absorbed slowly from the plasma into the white blood cells (42) and so the best diluent for the measurement of leucocyte ascorbic acid is that which allows an effective separation of the bulk of the red blood cells from the white blood cells in the shortest possible time. The guinea pig buffy layer does not separate in the dextran solution used for human L.A.A. estimations because of the differences in density of the blood cells in guinea pig and human blood.

In order to find the optimum molecular weight dextran for separating the guinea pig buffy layer, a range of dextran solutions were tested. The dextrans tested were:
The latter ($T_{125}$) is used for separating the buffy layer of human blood. The solid dextran were supplied by Pharmacia (Uppsala, Sweden). The solutions were all prepared in the same way viz. with 200 ml of 0.9% saline/50 ml of a 6% solution of the dextran in 0.9% saline/2 ml of 10% sequestrene in 0.9% saline.

A 3 ml aliquot of fresh blood, obtained by direct cardiac puncture from several guinea pigs and then pooled, was added to 12.5 ml of each dextran solution. The blood was allowed to settle for thirty minutes. There was no visible separation in the tubes containing $T_{10}$, $T_{40}$ and $T_{125}$ solutions but there was a very clear separation in the tubes containing $T_{250}$ and $T_{500}$ solutions. At the end of thirty minutes haematocrit measurements were taken from each tube. In guinea pig blood, the normal ratio of white blood cells to red blood cells is approximately 1:500. The ratio after separating the blood in $T_{500}$ is approximately 1:1. After separation in $T_{250}$, the ratio is roughly 1:2.

Therefore, from the visual observations and the haematocrit results, the optimum molecular weight dextran for separating guinea pig blood cells is $T_{500}$. The separation is complete in thirty minutes.
After separation in 12.5 ml of the T\textsubscript{500} dextran / saline / sequestrene solution, the supernatant layer is used for guinea pig leucocyte ascorbic acid estimations. The procedure and calculation is then exactly the same as for the estimation of L.A.A. in human blood (Chapter III, page 30).

**Estimation of vitamin C levels in guinea pig tissues.**

The vitamin C content of the liver, stomach and upper small intestine of guinea pigs are all measured in the same manner.

The tissue, 1 g, is homogenised in a piston homogenizer with distilled water (2 ml). 19 ml of distilled water are added to 1 ml of the homogenate. Then, equal volumes of the diluted homogenate and 10\% trichloroacetic acid (T.C.A.) are mixed together in order to denature the protein in the tissue and allow all the ascorbic acid to be dissolved.

Thus, the final concentration of T.C.A. in the solution is 5\% and the final dilution of the tissue is 1 : 120. At this stage, the estimation can be left overnight at 2-4\textdegree C.

The solutions are then centrifuged to remove cell debris and 1 ml of the resulting supernatant fluid is incubated with 0.3 ml of 100 vols of 2.2 g of 2,4 dinitrophenyl hydrazine in 10 N sulphuric acid / 5 vols of 5\% thiourea in distilled water / 5 vols of 0.6\% copper sulphate reagent for four hours at 37\textdegree C. After cooling in ice-water, 1.5 ml of 65\% sulphuric acid are added and the absorbance is read, against a reagent blank, at 520 mu
in a spectrophotometer.

A calibration graph is prepared using concentrations of 0.0, 0.5, 1.0, 2.5, 3.0, 4.0 and 5.0 µg of ascorbic acid / ml in 5% T.C.A., these being treated identically to the test solutions. The test readings can then be read off the straight line graph thus obtained.

The ascorbic acid content of the tissues is expressed as µg of ascorbic acid / g of wet weight of tissue and is calculated as follows:

\[
\text{Conc.(graph reading) } \times \text{ diln.} = \text{µg of ascorbic acid/g wet wt.}
\]

If the percentage water content of the tissues is known, the result can be expressed as µg of ascorbic acid / g dry weight of tissue and is calculated:

\[
\frac{\text{Conc.(graph reading) } \times \text{ dilution}}{(1 - \% \text{ water content})} = \text{µg/g dry wt.}
\]

Results.

The physiological conditions of the guinea pigs fed the scorbutic diet deteriorated during the three weeks of the experiment. There were no signs of scurvy after one week and the guinea pigs were normally active. There was however, some loss of coat hair especially about the nose. After two weeks, the animals still looked healthy but they had lost a lot of coat hair. At the end of the third week, the animals were rather inactive and they had lost weight and a lot of coat hair.

The normal range of vitamin C levels, expressed as µg/g wet weight, in the various guinea pig tissues is shown in Table 4.3. The percentage water content of
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Normal range of ascorbic acid (µg/g of wet wt. of tissue) (mean ± S.D.)</th>
<th>% water content (mean ± S.D.)</th>
<th>Normal range of ascorbic acid (µg/g of dry wt. of tissue) (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>265.8 ± 108.9</td>
<td>73.0 ± 1.2</td>
<td>985.9 ± 361.3</td>
</tr>
<tr>
<td>Stomach</td>
<td>123.5 ± 19.5</td>
<td>79.9 ± 1.3</td>
<td>614.8 ± 86.6</td>
</tr>
<tr>
<td>Upper small intestine</td>
<td>145.5 ± 19.9</td>
<td>81.4 ± 3.4</td>
<td>785.6 ± 107.4</td>
</tr>
</tbody>
</table>

**TABLE 4.3.** Ascorbic acid levels in various guinea pig tissues.
the same guinea pig tissues is also shown in this Table. These percentages were used to calculate the range of ascorbic acid levels in the dry weight of the guinea pig tissues (Table 4.3).

The leucocyte ascorbic acid levels showing the decrease in the levels of vitamin C when the guinea pigs were fed the scorbutic diet for a maximum of three weeks are shown in Fig. 4.1. There is no significant change in the guinea pig L.A.A. levels beyond the first week. The straight line graph obtained from these results is drawn in Fig. 4.1.

The levels of ascorbic acid in the guinea pig stomach decreases rapidly when the scorbutic diet is the only food available to the animals (Fig. 4.2). As with the L.A.A. results, there is no significant change in the levels after the initial decrease which occurred in one week. The vitamin C levels in the stomach decrease exponentially with time (Fig. 4.2). The liver ascorbic acid levels showing the onset of sub-clinical scurvy in guinea pigs are shown in Fig. 4.3. There is a significant change in the ascorbic acid levels after each week. The exponential curve \(y = ae^{bx}\), obtained from these results, is also shown in Fig. 4.3. The decrease in the vitamin C levels found in the upper small intestine (Fig. 4.4) mirrors the results obtained for the liver. The decrease is again exponential (Fig. 4.4).
Fig. 4.1: Leucocyte ascorbic acid levels, (μg/10⁸ WBC; mean ± S.D.) showing the onset of sub-N.S. clinical scurvy in guinea pigs (39 animals) fed the scorbutic diet.

 LINE: \( y = mx + b \)

\( r = -0.5 \)

\( p < 0.0005 \)
Fig. 4.2  Stomach ascorbic acid levels (μg/g wet weight: mean ± S.D.) showing the onset of sub-clinical scurvy in guinea pigs (21 animals) fed the scorbutic diet.

μg/g wet wt.

CURVE: \( y = ae^{bx} \)

- \( p < 0.0005 \)
- \( p < 0.0005 \)
- N.S.
- N.S.
Fig. 4.3 Liver ascorbic acid levels (μg/g wet weight: mean ± S.D.). To show the onset of sub-clinical in guinea pigs (21 animals) fed a scorbutic diet.

μg/g wet wt.

CURVE: \( y = ae^{bx} \)

\( r = -0.838 \)

\( p < 0.0005 \)
Fig. 4.4  Upper small intestine ascorbic acid levels (µg/g wet wt.: mean ± S.D.).

To show the onset of sub-clinical scurvy in guinea pigs (16 animals) fed a scorbutic diet.

\[ y = ae^{bx} \]

\[ r = -0.821 \]

\[ p < 0.0005 \]
Discussion.

The vitamin C levels in the tissues depend on dietary intake and the rate of absorption. Thus there is a wide variation in the tissue levels of ascorbic acid. The liver is richer in vitamin C than the stomach and the upper small intestine. This was expected since vitamin C is essential for several reactions which occur in the liver such as the formation of the highly reactive semidehydro-ascorbic acid (15). However, vitamin C has no known function in the stomach or upper small intestine. These differences in vitamin C levels also occur in the dried tissues.

Both the physiological results and the tissue levels indicate that sub-clinical scurvy develops when the guinea pig has been fed this scorbutic diet for two weeks. Frank scurvy will develop in about three weeks. By contrast, man requires three to four months to show symptoms of scurvy. This difference is due to the fact that in man, ascorbic acid has a half-life of sixteen days whereas in the guinea pig the half-life of ascorbic acid is only four days (27). Also in man, about 1.0 mg/kg/day is required to maintain a body pool of 20 mg/kg, but in guinea pigs an average of 9.3 mg/kg/day are required to maintain a body pool of 54 mg/kg. Therefore, a smaller dose of ascorbic acid, when compared on an equivalent weight basis, is required to maintain the body pool of the vitamin in man than in the guinea pig.

Equilibrium between ascorbic acid absorbed from the diet and that present in the tissues is reached in less
than four hours. Leucocyte ascorbic acid levels parallel tissue levels and although there is a wide variation in these results, they clearly indicate the onset of sub-clinical scurvy in guinea pigs. Tissue levels, especially liver levels, are also a sensitive index of the vitamin C status of the animal.

Thus these quantitative measurements in the blood and tissues are used in future experiments to define the sub-clinically scorbutic state in guinea pigs.
The levels of alcohol dehydrogenase in sub-clinically scorbutic guinea pigs.

It is an old observation that alcoholics have an upper limit of tolerance which is usually three to four times the amount of alcohol that can be consumed by the occasional drinker. The reason for this elevated biochemical tolerance has often been sought in an adaptive increase in the enzymatic processes involved in the oxidation of alcohol. Some recent studies (47, 48) have been concerned with the effect of chronic alcohol administration on the activity of liver alcohol dehydrogenase (ADH) in experimental animals. The data obtained from these studies are somewhat at variance with each other. Reports of in vitro studies on ethanol oxidation or activity of ADH and catalase in the liver of rats pretreated with ethanol have also been contradictory (49).

Nutrition affects the rate of ethanol metabolism. It has been shown that the rate of ethanol metabolism is diminished in fasted animals (50). Many experiments, designed to study the biochemical effects of alcohol, have been completed using laboratory animals. In these experiments, the animals are usually fed a well defined standard diet with all the supplements of vitamins and trace metals. However, the results of these experiments should be applied with caution since the diet consumed by alcoholics is very varied and often inadequate in some essential components.

Since it has been shown that, as a group,
alcoholics are sub-clinically scorbutic (Chapter III),
attentions were made to discover if this condition could
directly affect their metabolism of ethanol. Thus an
experiment was designed to measure the levels of alcohol
dehydrogenase (ADH) in sub-clinically scorbutic guinea
pigs.

The metabolic pathway of ethanol oxidation in
guinea pigs is the same as that in man. Ethanol is
converted to acetaldehyde in the presence of ADH with
nicotinamide adenine dinucleotide (NAD) as an essential
cofactor. ADH is the first enzyme and is the rate
limiting enzyme in this metabolic pathway. ADH is
induced in the liver when alcohol is taken.

Materials and methods.

Only male guinea pigs, weight 240-450 g, were
used in these experiments.

Experiment 1.

Sixteen male guinea pigs were divided into two
groups each comprising eight animals. One group was
given diet 18 and cabbage and the other was given the
scorbutic diet (Table 4.2). Both groups were given tap
water as their sole drinking fluid. The guinea pigs were
caged individually and were given fresh aliquots of food
and liquid every day. They were maintained in these
groups for twelve days. On the next two consecutive days,
six animals from each group were injected intraperitoneally
with 2 ml of 30% ethanol (Burrough's 95% ethyl alcohol)
in saline (v/v) and given 10% ethanol in tap water as
their sole drinking fluid. At the end of two weeks, the
guinea pigs were killed by cervical dislocation and the liver rapidly excised for the measurement of ADH by the method described overleaf. The guinea pigs were injected with alcohol on the last day of the experiment and were killed 90 minutes from the time of injection.

A blood sample was taken from all the water drinking animals and half of the alcohol drinking animals for L.A.A. estimation by the method described in Chapter IV. The part of the liver not used for ADH measurements was taken from half of the guinea pigs and stored at -70°C until the liver ascorbic acid levels were measured by the dinitrophenyl method described in Chapter IV.

**Experiment 2.**

This experiment was conducted exactly as above but the guinea pigs were maintained in the first groups for only one week. During the second week, the animals were split into four groups, since six animals in each of the original groups were injected daily with ethanol and given ethanol as their sole drinking fluid in the same doses as in Experiment 1. At the end of the second week, all the guinea pigs were killed by cervical dislocation and the liver was rapidly excised for the measurement of ADH.

Blood samples were obtained for L.A.A. measurements and the livers were stored for liver ascorbic acid measurements as before.

**Experiment 3.**

Sixteen male guinea pigs were divided into the following four groups:
The experiment was carried out as before. The guinea pigs in the alcohol groups were injected intraperitoneally with 2 ml of 30% ethanol in saline daily for two weeks. At the end of this period, ADH levels were measured in all the guinea pigs, blood samples were obtained for L.A.A. estimations and livers were stored from half the guinea pigs for ascorbic acid measurements. Experiment 4.

In this experiment, sixteen male guinea pigs were split into two groups with eight animals in each. All the animals were fed diet 18 and given cabbage every day. Four animals were given water as their sole drinking fluid while the other twelve were given 10% ethanol in tap water. The latter group were injected daily with 2 ml of 30% ethanol in saline. The guinea pigs were maintained in these groups for one week. After this, the guinea pigs were divided into the four groups described in Table 5.1, and the animals receiving alcohol were injected daily. The guinea pigs were maintained in these

<table>
<thead>
<tr>
<th>Food</th>
<th>Liquid</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Diet 18 + cabbage</td>
<td>Tap water</td>
<td>2</td>
</tr>
<tr>
<td>2. Diet 18 + cabbage</td>
<td>10% ethanol in tap water (v/v)</td>
<td>6</td>
</tr>
<tr>
<td>3. Scorbutic diet</td>
<td>Tap water</td>
<td>2</td>
</tr>
<tr>
<td>4. Scorbutic diet</td>
<td>10% ethanol in tap water (v/v)</td>
<td>6</td>
</tr>
</tbody>
</table>
four groups for two weeks. At the end of three weeks, the ADH levels in the liver were measured along with the L.A.A. and the liver ascorbic acid levels.

**Experiment 5.**

Two dozen male guinea pigs, weight range 234-275g, were divided into the same groups as described in Table 5.1. There were six animals in each group. The guinea pigs in the alcohol drinking groups were injected intraperitoneally with 2 ml of 30% ethanol in saline. The animals were maintained in these groups for two weeks. At the end of two weeks, the guinea pigs were killed by cervical dislocation and the livers rapidly excised for the measurement of nicotinamide adenine dinucleotide (NAD) by the method of Jedeiken and Weinhouse (51).

**Method for the measurement of ADH in guinea pig liver.**

The guinea pig is killed by cervical dislocation and the liver is rapidly excised. The liver sample, 1 g, is homogenised with 3 ml of 0.1M sodium pyrophosphate buffer (pH = 9.0) in a piston homogeniser. The homogenate is then sonicated for exactly one minute at medium power and amplitude 4 using an M.S.E. 10 k.watt ultrasonic disintegrator. Sonication breaks down the cell walls and allows all the ADH to be released from the cell. After sonication, the samples are spun down at 10,000 g at 4°C for thirty minutes to remove most of the cell debris and then the supernatant fraction is re-spun at 100,000 g at 4°C in an ultracentrifuge for one hour (52). All the ADH is contained in the clear supernatant fraction.

The levels of ADH are estimated indirectly on a
spectrophotometer (Pye-Unicam S.P. 8000). The increase in optical density at 366 m\(\mu\) indicates the rate of formation of reduced nicotinamide adenine dinucleotide (NADH), which depends quantitatively on the amount of ADH in the cell. The determination of the activity depends on the reaction:

\[
\text{ADH} \quad \text{C}_2\text{H}_5\text{OH} + \text{NAD} \quad \xrightarrow{\text{CH}_3\text{CHO} + \text{NADH} + H^+} 
\]

In alkaline solution, and by trapping the acetaldehyde with semicarbazide, the equilibrium is shifted almost quantitatively to the right. The assay mixture contains:

- 0.1M sodium pyrophosphate buffer 2.57 ml
- 2.2M semicarbazide solution 0.10
- 96% ethanol 0.10
- 0.03M NAD solution 0.20
- 0.3M glutathione solution 0.01

This solution is mixed and the reaction is started by the addition of 0.02 ml of the supernatant, which is the enzyme containing solution. The solution is again mixed and the optical density at 366 m\(\mu\) and 25°C is read every minute for five minutes. The mean change in extinction (\(\Delta E/\text{min}\)) is calculated from these readings. The calculation is completed as follows:

\[
\frac{\Delta E/\text{min.V.}}{\epsilon \text{ d.v.}} = \text{Units/ml enzyme sample solution.}
\]
Where:

\[ \Delta E/\text{min} = \text{optical density difference/minute}. \]
\[ V = \text{assay volume in ml. (3 ml)}. \]
\[ E = \text{extinction coeff. of NADH (3.3cm}^2/\mu \text{ at 366mu)}. \]
\[ d = \text{light pathway in cm. (1 cm)}. \]
\[ v = \text{sample volume in ml. (0.02 ml)}. \]

From this, it follows that:

\[ \Delta E/\text{min} \times 45.5 = \text{units/ml enzyme sample solution}. \]

and the specific activity is calculated:

\[ \frac{\Delta E/\text{min} \times 45.5}{\text{mg. protein taken}} = \text{units/mg. protein} \]

The protein content of the solution is measured by the Lowry method (54). One enzyme unit is the amount of enzyme which catalyses the transformation of 1 \( \mu \) mole of substrate per minute. (The abbreviation \( \mu \) is used to denote milli-unit).

Results.

ADH was maximally induced in guinea pigs fed ethanol within two days. The levels of alcohol dehydrogenase in the groups fed alcohol with either diet 18 or the scorbutic diet did not differ significantly beyond two days (Fig. 5.1). Consequently, all the results were pooled and only four groups were considered. Figure 5.2 shows the ADH levels in each of the collective groups.

The NAD levels measured in the guinea pig livers are shown in Fig. 5.3. The levels of L.A.A. measured in guinea pig blood and the liver ascorbic acid levels are shown in Table 5.2.
<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>L.A.A. levels (µg/10⁶ WBC; mean ± S.D.)</th>
<th>No. of animals</th>
<th>Liver ascorbic acid levels (µg/g; mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water + Diet 18</td>
<td>10</td>
<td>43.84 ± 17.06</td>
<td>6</td>
<td>148.25 ± 41.92</td>
</tr>
<tr>
<td>Alcohol + Diet 18</td>
<td>13</td>
<td>45.50 ± 15.50</td>
<td>10</td>
<td>184.50 ± 53.88</td>
</tr>
<tr>
<td>Water + Scorbatic diet</td>
<td>12</td>
<td>15.73 ± 5.26</td>
<td>6</td>
<td>38.80 ± 6.68</td>
</tr>
<tr>
<td>Alcohol + Scorbatic diet</td>
<td>11</td>
<td>12.31 ± 5.6</td>
<td>10</td>
<td>51.13 ± 12.30</td>
</tr>
</tbody>
</table>
**Fig. 5.1.** ADH levels in guinea pigs given 2 ml of 30% ethanol in saline daily and 10% ethanol as their sole drinking fluid, for different times (6 animals each group).

m. units of ADH/mg protein.

---

Guinea pigs fed scorbutic diet.

Guinea pigs fed diet 18.

No. of daily ethanol injections.
Fig. 5.2  ADH levels in guinea pig livers

m. units of ADH
/mg protein.

13
12
11
10
9
8
7
6
5
4

\[ p < 0.0001 \]

\[ \text{N.S.} \]

\[ p < 0.0005 \]

\[ p < 0.0005 \]

\[ p < 0.0005 \]

\( \begin{align*}
\text{H}_2\text{O + ND} & : \text{Tap water with diet 18.} \\
\text{EtOH + ND} & : 10\% \text{ ethanol and ethanol injected with diet 18.} \\
\text{H}_2\text{O + SD} & : \text{Tap water with scorbutic diet.} \\
\text{EtOH + SD} & : 10\% \text{ ethanol and ethanol injected with scorbutic diet.}
\end{align*} \]
Fig. 5.3 The levels of NAD in guinea pig liver.
The animals which were receiving ethanol were injected daily for two weeks with 2 ml of 30% ethanol in saline and given 10% ethanol in tap water as their sole drinking fluid.
Discussion.

Under these conditions of ethanol feeding, ADH is maximally induced in guinea pigs within two days. The levels of L.A.A. and liver ascorbic acid show that the guinea pigs fed the scorbutic diet are sub-clinically scorbutic. Thus there is a dramatic and highly significant increase in the ADH levels of sub-clinically scorbutic guinea pigs fed ethanol. Since hepatic metabolism is by far the most significant mechanism for the elimination of ingested ethanol in both man and guinea pig, an increase in such an important enzyme could partly explain the tolerance of alcoholics for alcohol.

Since ADH is the rate limiting enzyme in the pathway, it has been presumed that factors which affect the rate of ethanol metabolism in vivo do so through alterations in the rate of the initial oxidation reaction. The main factors which determine the rate of the ADH-catalysed dehydrogenation of ethanol are the concentration of the substrate, the level of ADH and the availability of NAD. The concentration of the substrate influences the rate of this reaction only at levels below which the enzyme is 'saturated'. It has been shown in dogs that the rate of ethanol metabolism is proportional to the concentration of ethanol in blood at levels below 10 mg%. Above this concentration, saturation of the enzyme occurs and the rate of ethanol metabolism is independent of the ethanol concentration (56). Since ADH is saturated at such low substrate concentrations, the availability of the enzyme becomes most important in the determination of the rate of
ethanol dehydrogenation at ethanol concentrations usually employed in animal and human studies. Thus, in sub-clinically scorbutic states, where the levels of ADH are increased, the rate of alcohol metabolism will be significantly increased.

The preceding statement is likely to be true only if one assumes an optimal supply of NAD to participate in the dehydrogenation reaction. With the oxidation of ethanol to acetaldehyde and the formation of acetyl CoA from acetaldehyde, NAD is reduced to NADH. If the supply is not to be exhausted, NADH must be constantly re-oxidised to NAD via some other oxidation-reduction reaction. It is known that there is some disparity between the rates of production of NADH and its oxidation to NAD as evidenced by the increase in the ratio NADH : NAD which occurs in the liver during ethanol metabolism (57). Since the sum of NAD and NADH is always constant in a cell, the results in this study, that is, a decrease in NAD levels when ethanol is injected, also indicate such a disparity.

Thus, metabolic systems involved with the oxidation of hepatic NADH may secondarily affect the rate of ethanol metabolism by influencing the availability of the oxidised co-factor. Dehydroascorbic acid is reduced to ascorbic acid in several reactions which occur in the cell. During this reaction, NADH is re-oxidised to NAD (Fig. 5.4). In the sub-clinically scorbutic state the amount of NAD present in the cell is increased. Thus, in sub-clinical scorbutic states, more NAD is available for alcohol metabolism. The results show that when ethanol is fed to
the sub-clinically scorbutic animal, there is a significant increase in the level of NAD (p < 0.0005). Thus, this whole cycle is encouraged by a lack of ascorbic acid and it has already been shown that alcoholics do lack ascorbic acid.
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