Ethanol
Endogenous

Endogenous Ethanol: A Review[1]

David Lester, Ph.D.

ON SOME 60 occasions since 1858 (1), various investigators have interested themselves in the question of the normal occurrence of ethanol in man under a variety of circumstances. Ethanol has been found in concentrations not differing from the "zero" of a particular analytical method to as high as 200 mg. per liter of blood. At times it seemed certain that ethanol did arise endogenously; at other times grave doubt was cast on the occurrence of even the low levels reported. Between those who believed in the occurrence of endogenous ethanol and those who did not, no disagreement is evident as to the relative unimportance of this particular contribution to the energy economy of the body. On both sides of this issue, no one attempted to assess the caloric contribution; all assumed that it must be trivial.

The general agreement in the literature over the constant rate at which ingested ethanol is eliminated, the few opposing views notwithstanding, would seem to offer support for those who saw little real evidence of the endogenous occurrence of ethanol. If ethanol is indeed eliminated in such a manner, the maintenance of any particular concentration, no matter how small, requires the liberation or formation of ethanol within the body in ridiculously high amounts; hence a linear substantial rate of ethanol disappearance tends to confirm the nonexistence of endogenous ethanol or, at the most, its trivial and negligible character. None of those who found more than traces of ethanol and believed ethanol to be formed endogenously concerned themselves with the consequences of their findings in terms of the rate of ethanol formation needed to produce the concentrations found. It must be concluded that all believed this rate to be insubstantial. They tended to investigate how the ethanol level may be changed, skirting the quantitative issue of rate of formation, and essentially agreed with the charge leveled against

[1] From the Laboratory of Applied Biodynamics and Center of Alcohol Studies, Yale University, New Haven, Conn.
Received for publication: 6 June 1961.

Quart. J. Stud. Alc. 22:555-574
1961

them that the normal levels they found were not all ethanol but some material or materials reacting as such. Any change in the level as a result of experimental procedure, however, was deemed to be ethanol.

Present opinion seems divided between a belief (*a*) that any ethanol found may be an artefact and does not arise endogenously and (*b*) that its presence is real, not artefact, but in any case is an insubstantial and trivial component of animal processes.

The topic divides naturally into two parts: (*1*) the rate of disappearance of ethanol at low levels; and (*2*) whether and in what amount ethanol is present in the tissues. The former aspect will be dealt with first, to indicate the importance of establishing the normal endogenous concentration.

Ethanol Disappearance at Low Levels

Data on the elimination of ethanol at concentrations below 100 mg. per liter of blood are available from only two sources, the first being an investigation of the ethanol levels attainable by inhalation (2). There, a uniform intake of small amounts of ethanol was established and serial analyses of the increased levels of ethanol were made. In two of three such experiments with a subject weighing 83 kg. a mixture of ethanol in air was respired at a concentration of 15 mg. per liter and at a rate of 15 liters per minute. Since it had previously been established that 62 per cent of the inspired amount was retained, the inhalation thus resulted in an intake of 139 mg. of alcohol per minute ($0.62 \times 15 \times 15$). If it is assumed that 62 per cent of all the body tissues and 93 per cent of the serum are water, and that alcohol is distributed uniformly throughout the body water (all valid assumptions), then an intake of 139 mg. of alcohol per minute is equivalent to 151 mg. per liter of serum per hour. At this rate of intake an equilibrium concentration of about 80 mg. of ethanol per liter of blood (equal to 90 mg. per liter of serum) was actually established in 2 hr. In the third experiment the subject was exposed to a concentration of 16 mg. of ethanol per liter of inspired air at a ventilation rate of 7 liters per minute, so that the intake was equivalent to 76 mg. per liter of serum per hour and the equilibrium concentration attained was about 45 mg. per liter of serum. When the concentration of ethanol in the serum does not change, its rate of disappearance equals its rate of intake, the rate of intake corresponding exactly to the decline as the re-

sult of metabolism and other losses. In this subject, the rate of disappearance, in milligrams per liter of serum per hour, is 1.68 times the equilibrium level, in milligrams per liter of serum, that is, 151 ÷ 90 and 75.5 ÷ 45. If, at these levels, the rate is proportional to the concentration—that is, a reaction of the first order obtains, as seems to be the case—then the concentration at which the maximal rate will be achieved may be obtained by dividing the known maximal rate of ethanol elimination, 225 mg. per liter of serum per hour, by 1.68. The quotient, 134 mg. per liter, is the concentration above which ethanol elimination becomes linear and constant and independent of concentration; this value, 134 mg. per liter, is in good agreement with a similar value obtained directly by Newman and Cutting (3).

In the second such investigation, Marshall and Fritz (4) studied the metabolism of ethyl alcohol in dogs directly at these low concentrations by serial analyses of serum when distribution of the ethanol in the body water was completed after intravenous administration of ethyl alcohol. In this manner they observed that the linear disappearance of ethanol became exponential at levels below 100 mg. per liter; at the lowest levels the rate of decline was proportional to the concentration; this rate, in milligrams per liter of serum per hour, is computed from their data to be 1.77 times the level in the serum, in milligrams per liter. Since the average maximal decline was about 135 mg. per liter per hour, this rate becomes maximal at levels above 75 mg. per liter (135 ÷ 1.77).

The agreement between these two sets of observations as to the exponential character of the decline and its magnitude is excellent.

Using the value 1.68, obtained in man, the rate at which endogenous ethanol would have to be formed may be computed for any level of ethanol. For the maintenance of each incremental equilibrium level of 10 mg. of ethanol per liters of serum, there is required, in a 70-kg. man, the formation of 0.79 g. of ethanol per hour [(10 × 1.68 × 70 × 0.62) ÷ 0.93], an amount yielding 5.5 calories or 7 per cent of the basal energy needs.

It is a matter of some importance, therefore, to know the concentration of ethanol circulating in the body, because its magnitude, and hence its contribution to the basal metabolic processes of the body, is determined by the concomitant rate of formation of ethanol.

Problems of Alcohol Analysis

A variety of adequate techniques are available for the identification and quantitative determination of the concentrations of ethanol which are of importance in forensic medicine or have significant effects upon physiological function. In many instances and for many purposes characterization is never at issue, either because the likelihood of another volatile reducing compound in relatively high concentration is nil or because it is known that ethanol was ingested. These analytical methods may also be adapted for the analysis of very low concentrations of ethanol. Theoretically, the same principles of analysis apply whether the concentrations of interest are high or low; practically, these principles must be observed in toto, and none may be disregarded, when the concentrations of interest are trifling from the viewpoint of any overt physiological activity, and particularly when the issue of their very existence is in question. A brief outline of the criteria by which to judge the validity of the values reported is therefore pertinent.

Values for ethanol that are less than the true value may arise as the result of inadequate recovery of ethanol present or by its destruction or loss prior to analysis. Such a contingency is met by calibrating a particular technique through the addition of known amounts of ethanol to the tissues to be analyzed and showing adequate precision or reproducibility in the recovery of the added ethanol. Most investigators have adhered to this criterion. Although the results obtained in this field are more subject to criticism on the opposite basis, that is, for being higher than the true value, it is well not to overlook the fact that values for ethanol near zero concentration may be just as much in error by underestimation as by overestimation.

Second only to water, ethanol, as the 95 per cent by volume solution with water, is the most common laboratory solvent. Its omnipresence demands the exercise of every care to insure its rigid exclusion from samples that are to be analyzed for ethanol. Although the requirement is obvious, it is not certain that it has always been met: substantial behavioral changes should have been observed if some of the reported concentrations of ethanol even approximated the true value.

Besides ethanol in the liquid phase as a contaminant, any air in direct contact with 95-per-cent ethanol liquid has a concen-

tration of about 100 mg. of ethanol per liter at 20°C. The absorption of 1 ml. of this atmosphere by a 10-ml. sample suffices to give a sample concentration of 10 mg. per liter. Nonexposure of samples to such atmospheres and the exclusion of ethanol are a sine qua non.

Most chemical methods for the determination of ethyl alcohol depend on a proportional reduction of some reagent by the ethanol, the reduction being nonspecific for ethanol. The specificity is increased considerably by separating ethanol from the blood or tissue by aeration, single or double distillation, diffusion, or by lyophilization from the frozen sample. If the sample is not analyzed immediately, the content of reducible material (not necessarily ethanol) available from a single distillate doubles in 24 hr. even if the sample is kept at refrigerator temperatures (5). The reducing material presumably arises as the result of the thermal decomposition of unknown compounds which continue to be formed in various tissues on standing. This may be obviated by immediate analysis of the samples, by keeping the samples frozen at —60°C, and by more than one distillation. Separation by lyophilization from the frozen sample and subsequent analysis of the lyophilate is the method of choice, but has thus far been used by only one group (6).

Problems of interference and specificity, accuracy and precision, associated with analysis for ethanol, have been ably reviewed recently by Lundquist (7). It is to be expected that the specificity of an enzymatic method would be higher than one not employing an enzyme. In addition to ethanol, alcohol dehydrogenase reacts with C_3–C_5 alcohols, whereas most reagents like dichromate and permanganate suffer reduction by these and other compounds. Because the enzymatic method is more specific, values obtained with it would be expected to be lower, and nearer to the true value, than those obtained with a nonenzymatic method. Curiously enough, in a carefully conducted series of analyses, Marshall and Fritz (4) found lower values with a dichromate method than with the enzymatic method, although the reproducibility was better with the latter. In this instance, the ethanol concentration had been elevated as a result of ingestion of ethanol: determinations gave a value of 96.0±0.11 mg. per 100 ml. of serum by the enzymatic method whereas 16 determinations gave a value of 94.3±1.62 by a dichromate method in the same sample of serum. Because of the significant difference in the variance of the two methods, no legitimate *t* test of the means is possible. The reason for the higher value

with the enzymatic method, however, might be the presence of materials in serum which exert greater interference with the enzymatic than with the dichromate method, so that even though a greater variety of structures interfere with the latter method, their absence in serum or in the sample separated from the serum may actually make a nonenzymatic method less subject to this interference. Marshall and Fritz (4) performed the enzymatic analysis directly on the serum, while the dichromate oxidation necessarily was performed on volatile materials separated from the serum, and it is this difference which probably accounts for the lower value with the nonenzymatic method. Differences in this direction—that is, values greater in the enzymatic than in the nonenzymatic method—can occur only if efforts are directed to reduce the extent of interference by proper separation techniques and choice of a method. For the low concentrations likely to be encountered with endogenous ethanol, a preliminary separation and concentration of the alcohol from the serum or blood sample is required in any case because of the low absolute sensitivity of the enzymatic method, so that at low concentrations of ethanol the enzymatic method undoubtedly will yield values close to the true value. Usually, therefore, reported values by the enzymatic method are lower than those by the nonenzymatic (Table 1). Enzymatic analysis of doubly distilled serum or, better, of a serum lyophilate would seem to be a combination of choice.

Both categories of methods have their disadvantages on grounds of specificity, time involved, and low, albeit adequate, sensitivity.

These failings can be overcome at the present time by using gas-liquid partition chromatography; this is several magnitudes more sensitive than other methods and has a specificity and speed similarly unexcelled.

Characterization as Endogenous Ethanol

The isolation and separation, characterization and quantitative determination of ethanol is an essential step in the process of establishing it as an endogenous metabolite but it is, by itself, not a sufficient basis upon which to reach this conclusion. For it must be shown that the ethanol, if any, so found, has in reality been produced by endogenous processes. In essence, this requires evidence best obtained from a variety of sources. Ethanol must not, of course, be ingested. Values from 70 to 2,530 mg. per liter of blood have

TABLE 1.—*Normal Levels of Ethanol in Human Tissues*

Tissue	*Concentration**	*Number of Subjects*	*Investigator*
Brain	15.7	6,000	Gettler & Tiber (12)
Brain	4	7	Gettler et al. (11)
Liver	26	15	" "
Blood	42	7	" "
Urine	0.60–1.85	6	Harger & Goss (5)
Blood	0.00–0.27	4	" "
Blood	5	>1	Baglioni (13)
Blood	1–4	2	Friedemann & Klaas (14)
Blood	20–40	>1	Allodi & Daprà (9)
Blood	3–51	22	Aue (15)
Blood	30–50	>1	Cavett (16)
Blood	40–70	125	Jetter (17)
Blood	0–70	10	Blotner (18)
Blood	14,34	1	Platt & Webb (19)
Blood	90–200	10	Leonardi (20)
Blood	0–9	20	Saviano & Vacca (21)
Blood	1.4–18.7	10	Pansini & Mazzone (22)
Blood	0–23	16	" " (23)
Blood	1.6–12.4	11	Pansini & Casaula (24)
Blood	6–28	3	" " (25)
Blood	0.66	53	Jokipii (26)
Blood	9–27	3	Lester & Greenberg (2)
Blood	2.4†	19	Bücher & Redetzki (27)
Blood	<10†	4	Marshall & Fritz (4)
Blood	20–39	105	Cakl (28)
Blood	30	94	Zysk, Witkowski & Kaleta (29)
Liver	67†	1	McManus, Contag & Olson (6)

* Mean or Range in milligrams per liter or per kilogram.
† Enzymatic method.

been obtained (8, 9) in confirmed alcoholics, yet the investigators were apparently under the impression that these amounts arose endogenously; certainly, at the highest level found, the subject must have been quite willing to continue in the described "ethylically fasting" condition. To ensure that the ethanol found is endogenous in origin, and is not produced by fermentation from carbohydrate in the gut, two approaches have been used.

Taylor (10), in an experiment limited to one dog, removed the alimentary tract under nitrous oxide anesthesia, allowed 18 hr. for recovery by the animal and for disappearance of any ethyl alcohol that may have entered the body from the gut prior to its

removal, and then killed the dog. The muscles were rapidly removed and 3 kg. of the excised tissue were mixed with 9 kg. of water and a distillate of two-thirds volume collected; the process was repeated about 15 times to a final distillate of 25 ml. From the distillate was obtained 0.3 g. of a *p*-nitrobenzoate ester identical in melting point, mixed melting point and nitrogen content with ethyl *p*-nitrobenzoate. The amount of ethyl alcohol was thus about 24 mg. per kg. of fresh muscle, equivalent to 31 mg. per liter of serum.

The second approach to the problem of the endogenous nature of any ethanol found is that taken by McManus, Contag and Olson (6); these workers compared two groups of rats whose diets differed only by the presence of 2 per cent succinylsulfathiazole. There was no significant difference in the ethanol concentrations of the rat livers after 3 weeks of the diets ad libitum, so that either there is no contribution of ethanol by the bacterial flora or succinylsulfathiazole does not affect bacterial activity in a manner to inhibit ethanol formation in the gut. The authors conclude that "it is therefore unlikely that bacterial metabolites contribute significantly to the formation of endogenous ethanol." Rat liver was found to contain 52 mg. of ethanol per kg. (Table 2).

Except in the two experiments cited (6, 10) no investigators have attempted, by means other than exclusion of ethanol ingestion, to ensure that the ethanol measured actually originated endogenously. Those workers who have studied the effects of numerous variables upon the "normal" ethanol level may even have assumed that the original concentration denoted as ethanol was produced by fermentation in the alimentary tract or was another reducing agent but certainly implicitly assumed that any subsequent increase was ethanol resulting from the experimenter's manipulation.

Normal Endogenous Ethanol

In species other than the human, few workers have reported values of normal alcohol, a reflection of the relative ease with which samples may be obtained for this purpose in the human subject. Table 2 lists some of the values found. Those reported by McManus, Contag and Olson (6) were determined enzymatically on a lyophilate of the tissue.

The values of ethanol found in tissues from individuals with no obvious disease process have been listed in Table 1. Nearly all of these values have appeared since the excellent tabulations and

TABLE 2.—*Normal Levels of Ethanol in Animal Tissues*

Species, Organ or Tissue	*Concentration*°	*Investigator*
Oxen, blood	2–16	Ford (1)
Oxen, liver	400	"
Dog, muscle	24	Taylor (10)
Dog, brain	3.3	Gettler et al. (11)
Dog, liver	6.9	"
Dog, blood	13.7	"
Pig, brain	0.7	"
Beef, liver	0.9	Harger and Goss (5)
Beef, kidney	0.6	"
Dog, brain	0.83	"
Dog, liver	2.13	"
Dog, kidney	0.93	"
Dog, muscle	1.32	"
Dog, blood	0.09	"
Rat, liver	52†	McManus, Contag and Olson (6)
Rat, plasma	23†	"
Rat, kidney	11†	"
Rat, muscle	18†	"
Rat, heart	49†	"
Rabbit, liver	31†	"

° Milligrams per liter or per kilogram.
† Enzymatic method.

reviews of Gettler, Niederl and Benedetti-Pichler (11) and Harger and Goss (5). The latter discussed, and mostly discounted—for some of the reasons enumerated—values of ethanol reported prior to 1935. Because greater reliance may be placed on the results obtained by Gettler and his associates (11, 12), these values are included in the tables. Not included is the indefinite concentration of "practically zero" obtained by Dotzauer et al. (30) in 19 healthy as well as diabetic subjects; this may, however, be a circumlocution for the mean value of 2.4 mg. per liter reported previously by Bücher and Redetzki (27). For somewhat different reasons, the concentrations reported by Möllerström (31) and by Barthe et al. (8) are also not included; in these instances the levels found were so high that it must be concluded that in the former case the analytical method was at fault, and that in the latter the ethanol source was exogenous, as it might be when "ethylically fasting" alcoholics are among the subjects.

There would appear to be equally valid arguments for concluding

that the normal ethanol is less or greater than 10 mg. per liter. Nonbelief in the possibility of the formation of endogenous ethanol helps to discount the higher values; if lower values, even zero concentrations, are found, then it is concluded that anything higher is caused by accidental contamination, methodological error, or other flaws. Thus, because they themselves found trifling amounts of ethanol in animal and human tissues, Harger and Goss (5) were inclined to comment favorably upon a similarly low concentration of ethanol in pig brains, reported by Gettler, Niederl and Benedetti-Pichler (11), in the following manner: "This very low figure is in marked contrast to the other figures reported by these investigators using smaller quantities of tissues. Thus their maximum figure for human liver alcohol is *eighty times greater* than this figure for pigs' brains." That Gettler, Niederl and Benedetti-Pichler used 28 kg. of pig brains for their analysis of this tissue is hardly relevant to the concentration in human liver; obviously if the highest concentration in human liver is 56 mg. per kg. (80 times that in pig brains) 1/80 of 28 kg. of human liver will yield as much alcohol as that in pig brains. How much tissue is used depends on the concentration in the tissue and the sensitivity of the analytical method, and neither bears on the question of the validity of the ethanol level found. The argument for the virtual absence of ethanol overlooks the fact that some of the investigators who have reported values higher than 10 mg. per liter also reported concentrations less than 10 mg. per liter. It is interesting that most of the lower values have appeared when smaller numbers of individuals were tested, values greater than 10 mg. per liter appearing more frequently when larger groups were used.

Although Harger and Goss (5) believed the concentrations to be trifling, they recognized that "the so-called normal" ethanol might occur. Twenty years later, in 1956, apparently all doubt had been removed, for in response to a query, it was stated (32):

> "There is no normal blood alcohol level. Early investigators found reducing substances in the blood that were thought to be alcohol: however, methods more specific for alcohol fail to show this substance in the blood of normal individuals. A recently developed method for determining alcohol, in which alcohol dehydrogenase is used, is highly specific and shows no normal alcohol. The alcohol that is occasionally found can be traced to the ingestion of substances containing alcohol, or the alcohol may be inhaled or may even be absorbed through the skin, as could occur from an alcohol rub."

Unfortunately the years have not brought such certainty, nor must all those who actually have small concentrations of ethanol be deemed not "normal." Although with the alcohol dehydrogenase method Bücher and Redetzki (27) and Marshall and Fritz (4) reported concentrations below 10 mg. per liter, Paulus and Mallach (33) in a series of diabetic patients found blood levels from 0 to 60 mg. per liter. McManus, Contag and Olson (6) combined separation by lyophilization and analysis by the enzyme method and found 67 mg. of ethanol per kg. of human liver and, in consequence, a value in human serum substantially more than 10 mg. per liter. Like Gettler, Niederl and Benedetti-Pichler (11), McManus, Contag and Olson (6), the latter with a variety of techniques, characterized the object of investigation as actually ethanol. Thus, taking into account also the work of Taylor (10), the conclusion is inescapable that ethanol, not other similar compounds, can be present and can have an endogenous origin. That the extent to which it occurs may vary from person to person, and even from one time to another, is not occasion for surprise in biological work. Since the cause of the variation is as yet unknown, it is impossible to predict in whom and when higher or lower concentrations will be present; by the same token a substantial spread of individual values is more likely to occur when larger numbers of subjects are tested.

Effects of Various Body States

Presumably because endogenous ethanol might be formed in the course of the intermediary metabolism of carbohydrate, and more possibly when there are defects in this metabolism, several studies have involved diabetics as subjects. Pansini and Mazzone (22, 23, 34) found no evidence of any difference between the concentration of ethanol in the blood of healthy individuals and in that of diabetics; the same levels were also found in individuals with liver disorders. Mauro (35) found the endogenous ethanol concentration in the diabetic to be more variable than in the normal, while Jokipii (26) found its mean in the diabetic to be slightly higher. Möllerström (31) divided diabetics into groups based on absence or presence of the ability to form ethanol endogenously, but this is the result of serious deficiencies in analytical technique; Gutschmidt's results (36) show no such division among diabetic patients. Neither diabetes insipidus (18) nor cardiovascular failure

(25) produces changes in ethanol concentration. In a series of 62 pregnant women, Candela (37) found, in blood samples taken from the fasting subject, an increase during the third to ninth months to between 40 and 60 mg. of ethanol per liter; during labor the level went as high as 135 mg. per liter, but it was only 14 mg. per liter in the infant immediately after delivery. Russo (38) found levels between 90 and 100 mg. per liter in patients with nephritis, and in patients during the convulsions of puerperal eclampsia the level was between 250 and 300 mg. per liter.

Compared to concentrations ranging between 20 and 40 mg. per liter in fasted nonalcoholics, Allodi and Daprà (9) found between 40 and 70 mg. per liter in the blood of alcoholics; and in alcoholics with evident hepatic insufficiency the ethanol concentration ranged from 70 to 250 mg. per liter. The even more obvious contamination by exogenous sources, at least in certain alcoholics, is evident in the report of levels between 120 and 2,530 mg. per liter by Barthe et al. (8).

Effects of External Agents

The effect of various agents and conditions upon the normal level of ethanol has been investigated. The ethanol concentration is found to be increased by the administration of glucose (23, 34), insulin (24, 39, 40), adrenalin (21), cocarboxylase (41, 42) and adenosine triphosphate (43). Intravenous administration of 100 mg. of cocarboxylase (thiamin pyrophosphate) is the only material which produces consistent increases in the ethanol concentration; adenosine triphosphate, the probable phosphorylating agent for the conversion of the vitamin to the coenzyme, thus would act presumably to increase the ethanol level by increasing the supply of the coenzyme. But why and how this increase is brought about is unclear; and alcoholics, not particularly well supplied with this vitamin, apparently exhibit higher levels of endogenous ethanol than nonalcoholics. Fasting up to 48 hr. has little effect (44); the ethanol level oscillates, being lower in the evening than in the morning hours. A decrease in the ethanol concentration is apparently caused by blood letting (25), but only in healthy persons; the withdrawal of 250 ml. of blood from individuals with cardiovascular failure resulted in an increase of the ethanol concentration. The reported effects of these various regimens upon endogenous ethanol await confirmation by other groups of investigators.

That oxygen inhibits carbohydrate breakdown in vitro, an effect first noted by Pasteur, and that its absence increases the rate of ethanol formation, has led several investigators to attempt to reproduce this effect in the whole body. In 3 of 10 fasted humans exposed to oxygen concentrations equivalent to barometric pressures of 296 to 469 mm. Hg for 10 to 30 min., Leonardi (20) found a rise in the concentration of ethanol from between 90 and 200 mg. per liter to between 110 and 440 mg. per liter; in guinea pigs exposed to a pressure of 500 mm. Hg, 11 of the 12 fasted animals and 1 of 3 fed animals were found by the same investigator (45) to have increased the level of ethanol to between 200 and 1,100 mg. per liter. The highest of these values, as Leonardi points out, might indeed be implicated as the cause of mountain sickness, were these levels actually to exist. Saviano and Vacca (21) exposed 20 subjects, male and female, for 1 hr. to a reduced air pressure of 450 mm. Hg. The ethanol concentration rose in 14 of the subjects from a preexposure level between 0 and 9 mg. per liter to between 10 and 82 mg. per liter; an additional rise was produced by the administration of adrenalin in the 14 responsive subjects. In similar experiments Zysk, Witkowski and Kaleta (29) exposed 94 subjects either to flights at high altitude or to a decompression chamber. After 1 or 2 hr. at 3,000 to 4,000 meters (462 to 526 mm. Hg) the concentration of ethanol increased from 30 to 190 mg. per liter, while ½ hr. at 5,000 m. (405 mm. Hg) increased the level to 150 mg. per liter; the concentration was still elevated 2½ hr. after return to sea level. Exposure to an altitude of 10,000 m. (198 mm. Hg) produced no change if oxygen at sea level partial pressure (159 mm. Hg) was available.

The effect of an increase rather than a decrease of the oxygen partial pressure above the normal 159 mm. Hg at sea level upon the normal ethanol concentration has not been investigated. If the effect should be to reduce the concentration, it is interesting to speculate about the relationship of this effect to the belief of some clinicians that the breathing of 100 per cent oxygen acts to prevent alcoholics from going on a binge: Is a low resting ethanol level correlated with lack of craving for drink?

Unless it is believed that administration of glucose, insulin, thiamin pyrophosphate, or adenosine triphosphate, and the imposition of hypoxic conditions, can produce volatile materials other than ethyl alcohol, and in the absence of contradictory experimental data,

it is difficult to avoid the conclusion that these effects, by causing changes in the concentration of ethanol, give support to the view that ethyl alcohol arises endogenously, and to a substantial extent.

Source of Endogenous Ethanol

The possible source of the endogenous ethanol has been studied by McManus, Contag and Olson (6), who speculated that its formation might result from the action of alcohol dehydrogenase, under reducing conditions, upon acetaldehyde arising from the action of pyruvic oxidase upon pyruvic acid. From radioactive pyruvate-2-C^{14} incubated anaerobically with liver slices, a conversion of 0.3 per cent to ethanol was obtained; the small yield raises a question of possible contamination, but indicates the importance of further study of this and other avenues for the formation of ethanol.

If ethanol is produced endogenously, then its further oxidation to acetaldehyde—a reversal of the pathway speculated upon by McManus, Contag and Olson (6)—should result in some ever-present concentration of acetaldehyde; that is, indeed, the case, the concentration of acetaldehyde being normally between 0.22 and 0.37 mg. per liter of blood (46). Jacobsen (47), however, was unable to find any significant rise in the blood acetaldehyde of the rabbit treated with disulfiram, an inhibitor of acetaldehyde oxidation, and therefore concluded that, in the rabbit, "very little, if any, acetaldehyde can be formed during normal metabolism." In the rabbit not treated with disulfiram, Jacobsen reported an acetaldehyde concentration of 0.00 to 3.0 mg. per liter and he believes the presence of acetaldehyde to be specifically linked with the metabolism of ethanol (48). If this is true, it is difficult to avoid the conclusion that acetaldehyde is present normally precisely because ethanol is a product of normal metabolism.

The administration of ethanol to man produces a more or less proportionate increase in the concentration of acetaldehyde, and from the work of Raby (49) it may be concluded that the acetaldehyde level is about 1/100 the level of ethanol. If this proportion, found with ingested ethanol, holds also for ethanol of endogenous origin, then multiplying Stotz's (46) values of the normal concentration of acetaldehyde in the human by a factor of 100 yields a normal endogenous ethanol concentration of between 22 and 37 mg. per liter. This computed result is of the same order as that

obtained by direct analysis for ethanol (Table 1) and seems hardly likely to be fortuitous.

Discussion

The proponents of the virtual absence of ethanol as an endogenous metabolite base their argument mainly upon their own findings of low and trifling amounts, and because of this finding raise the probabilities of contamination from exogenous sources of one type or another and nonspecificity of analytic methods to discount the higher values reported by other workers. Their criticism is probably valid in many instances. Not all the higher values reported are easily to be dismissed, however, in view of the fact that careful work and separation of ethyl alcohol from tissue by reason of its volatility leads to great specificity even with certain of the nonenzymatic methods. Also, some investigators used control subjects, in a sense; for both high and low values were found in different subjects, presumably equally treated experimentally. Additionally, various agents increased the ethanol level in particular subjects who thus acted as their own controls. These considerations tend to support the view of the occurrence of normal ethanol. The terms "virtual absence," "practically zero" or "so-called normal ethanol" refer to concentrations below 10 mg. per liter of serum; those who believe in this minimal occurrence admit that endogenous ethanol can occur, albeit in these small and trivial amounts. The point at issue thus appears to be the occurrence of ethanol in concentrations greater than 10 mg. per liter. If, in addition to reported ethanol values higher than this, note is made of the evidence from normal acetaldehyde levels, as well as the definitive work of McManus, Contag and Olson (6), then it would appear that concentrations of the order of 20 to 30 mg. per liter of serum may not be rare. Such concentrations would represent an important involvement of ethanol in the energy economy of the body.

The weight of the evidence from the literature indicates that ethanol occurs normally in mammalian tissue and that it is produced endogenously. Because its contribution appears to be both significant and substantial, it is important to establish its exact status in human beings of various ages and conditions.

A definitive reinvestigation of the subject would involve the determination of ethanol, its endogenous occurrence, the rate of its formation, and its origin in metabolism.

Analysis by gas-liquid partition chromatography of ethanol (50) separated by lyophilization from frozen tissue avoids the pitfalls of other approaches. An even simpler technique, useful especially in establishing the range of ethanol values in large numbers of humans, is the analysis of expired alveolar air: the air sample is, in effect, the result of aeration of the blood, the concentration of ethanol in the alveolar air being proportional to that in the blood. Although the concentration in the alveolar air is but 1/2,100 of that in the blood, the sensitivity of gas chromatography is so great as to make this determination feasible. These approaches appear ideal from the point of view of simplicity, speed and specificity. Additionally, the avoidance of possible sources of contamination, from within and without the sample, and scrupulous adherence to other tenets of the analyst's creed, will yield valid results, little subject to criticism.

The endogenous nature of ethanol can hardly be established by routine removal of the alimentary tract in the human or even by the simpler procedure of feeding succinyl sulfathiazole to suppress possible bacterial fermentation of carbohydrate; but simultaneous sampling at various levels of the gastrointestinal tract and blood or air is a means of resolving the dilemma in the human subject. The use of germ-free animals combines the definiteness of Taylor's method (10) and the simplicity of bacterial inhibition and has the virtue of utilizing physiologically intact organisms which may be studied over as long a time as necessary and under a variety of dietary and other circumstances.

The determination of the rate of disappearance of ethanol can be measured easily in the animal by the administration of amounts of radioactive ethanol (1 mg. per kg.) that will not appreciably alter the normal equilibrium concentration of ethanol. In the human, the measurement of the rate of disappearance of ethanol after intravenous administration of small amounts (0.1 g. per kg.) or the determination of the rate of intravenous administration of ethanol required to maintain an ethanol concentration above the resting level, will give the data required to compute the rate of endogenous formation at any given equilibrium concentration.

Study of the origin of endogenous ethanol should involve not only the in vitro approach (6) but a restudy in the whole organism of the effects of low and high oxygen, thiamin and other variables, including changes in the dietary.

Possible Relation to Alcoholism

I have suggested (51) that ethyl alcohol may have been at some time in the evolutionary past an important source of energy for living things. On this previous occasion, however, the major concern was with ethanol from exogenous sources. Rather than believe in the adventitious presence of alcohol dehydrogenase, the abundant presence of this enzyme, which occurs to the extent of about 0.15 per cent in mammalian liver, was felt to be a reflection of the past, alcohol dehydrogenase not being as yet completely selected out, perhaps because of its requirement in minor amounts for the transformation of Vitamin A. Another and more attractive explanation is that today, and not some time in the remote past of the race, some part of the normal intermediary metabolic pathways of various foodstuffs, such as carbohydrate, proceeds through ethanol, and the enzyme, besides making it possible to utilize ethanol as an energy source, also affords protection against the otherwise disturbing physiological effects of elevated and persistent concentrations. If this explanation should be correct, if ethanol is indeed a normal product arising endogenously in mammalian tissues, then it may be considered in a category different from other addictive agents, and its concentration and the rate of its formation may well be related to the liability which different individuals may have to alcohol addiction. In the previous communication, it was suggested that the individual capable of using acetate efficiently for synthetic purposes was not a likely candidate for alcoholism, while those not so capable might be alcoholism prone; because ethanol is apparently utilized more efficiently than acetate for synthetic purposes (52) the alcoholism-prone individual might satisfy his requirement for a 2-carbon source with ethanol. If, as seems likely, individuals vary in their endogenous concentration of ethanol, and if ethanol can be used efficiently for synthetic purposes by everyone, then four possible categories exist by the combination of efficient and inefficient acetate users and individuals with high and low rates of endogenous ethanol formation. I would not expect the individual who utilizes acetate efficiently and who also has a high rate of ethanol formation, and consequently the same rate of ethanol utilization, to be alcoholism prone; on the other hand, the combination of inefficient acetate use and relatively low rate of ethanol formation might result in an individual prone to alcoholism. The

normal presence of ethanol makes it possible to indulge in less tortuous speculations of the basis of craving than has been possible hitherto; it could be that this is reducible to the craving arising because ethanol is absent or that the craving is precipitated by a fall in the normal concentration of ethanol.

The elucidation in a quantitative manner of the status of ethyl alcohol as a normal endogenous metabolite will undoubtedly lead to more firmly grounded and less hazardous speculations and hence to a better understanding of the genesis of alcohol craving. It may also, conceivably, serve to advance the treatment of the alcoholic.

Summary

The literature on the status of ethyl alcohol as a normal endogenous metabolite has been reviewed.

Although many of the reported values are held to be clearly in error, because of one or another deficiency of technique, it is nevertheless concluded that (*1*) ethanol as such, and not another volatile reducing material, occurs in human beings and other mammals; (2) ethanol is formed endogenously and not as the result of bacterial fermentation in the intestinal tract; (3) the endogenous ethanol concentration varies from individual to individual; and (*4*) the concentration of ethanol may be increased in a number of ways, notably by hypoxia.

The rate of formation of ethanol at the low concentrations probably obtaining in the human requires the formation of 0.79 g. of ethyl alcohol per hour in the 70-kg. adult for each increment of 10 mg. of ethanol per liter of serum. As a normal level in human beings of between 20 and 30 mg. per liter seems not unlikely, 1.6 to 2.4 g. of ethanol may be produced per hour, accounting for some 14 to 20 per cent of the basal energy requirement.

Because of the bearing this may have on the addictive process, on the genesis of alcoholism and on the treatment of the alcoholic, some suggestions are made for a thorough investigation of this question to resolve all matters at issue with regard to the endogenous nature of the ethanol and its identification and concentration.

An hypothesis about alcohol craving is advanced on the basis of the occurrence of endogenous ethanol.

REFERENCES

1. Ford, W. H. Normal presence of alcohol in the blood. J. Elliot Soc. nat. Hist. I: 43–99, 1859. Cited in: Ford, W. H. On the presence of alcohol in normal blood and tissues and its relation to calorification. J. Physiol. 34: 430–443, 1906.
2. Lester, D. and Greenberg, L. A. The inhalation of ethyl alcohol by man. *I.* Industrial hygiene and medicolegal aspects. *II.* Individuals treated with tetraethylthiuram disulfide. Quart. J. Stud. Alc. 12: 167–178, 1951.
3. Newman, H. W. and Cutting, W. C. Alcohol injected intravenously: rate of disappearance from the blood stream in man. J. Pharmacol. 54: 371–377, 1935.
4. Marshall, E. K., Jr. and Fritz, W. F. The metabolism of ethyl alcohol. J. Pharmacol. 109: 431–443, 1953.
5. Harger, R. N. and Goss, A. L. The so-called normal alcohol of the body. Amer. J. Physiol. 112: 374–382, 1935.
6. McManus, I. R., Contag, A. O. and Olson, R. E. Characterization of endogenous ethanol in the mammal. Science 131: 102–103, 1960.
7. Lundquist, F. The determination of ethyl alcohol in blood and tissues. In: Methods of Biochemical Analysis; Vol. VII, Art. 7. New York; Interscience; 1959.
8. Barthe, R., Paris, J., Duchemin, M. and Thomas, F. Valeur et interprétation des taux d'oxycarbonémie obtenus par la méthode Nicloux-Eudiométrie. *Deuxième note.* Oxycarbonémie et intoxications exogènes: l'oxycarbonémie des fumeurs et des éthyliques. Arch. Mal. prof. 14: 288–290, 1953.
9. Allodi, A. and Daprà, L. L'importanza del tasso alcoolemico a digiuno per la diagnosi di insufficienza epatica. Arch. ital. Mal. Appar. dig. 6: 558–564, 1937.
10. Taylor, A. E. On the derivation of ethyl alcohol contained in the muscle. J. biol. Chem. 15: 217–220, 1913.
11. Gettler, A. O., Niederl, J. B. and Benedetti-Pichler, A. A. The isolation of pure, anhydrous ethyl alcohol from non-alcoholic human and animal tissues. J. Amer. chem. Soc. 54: 1476–1485, 1932.
12. Gettler, A. O. and Tiber, A. The alcoholic content of the human brain; its relation to intoxication. Arch. path. 3: 218–226, 1927.
13. Baglioni, A. [In: Foreign Letters—Italy. Reunion of gastro-enterologists.] J. Amer. med. Ass. 106: 2019–2020, 1936.
14. Friedemann, T. E. and Klaas, R. The determination of ethyl alcohol. J. biol. Chem. 115: 47–61, 1936.
15. Aub, E. Der Alkoholspiegel im Blut unter verschiedenen Bedingungen. In: Kionka, H., ed. Pharmakologische Beiträge zur Alkoholfrage, No. 8. Jena; Fischer; 1938.
16. Cavett, J. W. The determination of alcohol in blood and other body fluids. J. Lab. clin. Med. 23: 543–546, 1938.
17. Jetter, W. W. The chemical and clinical diagnosis of acute alcoholism. New Engl. J. Med. 221: 1019–1024, 1939.
18. Blotner, H. Alcohol tolerance tests in normal individuals and in patients with diabetes mellitus and diabetes insipidus. New Engl. J. Med. 220: 283–289, 1939.
19. Platt, B. S. and Webb, R. A. Fermentation and human nutrition. Proc. Nutrit. Soc. 4: 132–140, 1946.
20. Leonardi, G. Iperalcolemia endogena nell'uomo normale in condizioni di ipossia sperimentale. Boll. Soc. ital. Biol. sper. 23: 703–705, 1947.

21. Saviano, M. and Vacca, C. Variazioni dell'alcoolemia e della glicemia in seguito ad iniezioni di adrenalina confrontate con quelle da depressione barometrica in soggetti normali. Riv. Med. aeronaut. 11: 231–238, 1948.
22. Pansini, R. and Mazzone, R. Studi sull'alcolemia endogena; l'alcolemia artero-venosa nei normali. Boll. Soc. ital. Biol. sper. 27: 372–375, 1951.
23. Pansini, R. and Mazzone, R. Sui rapporti tra alcolemia e metabolismo glicidico: l'alcolemia dopo carico di glucosio nei soggetti normali. Boll. Soc. ital. Biol. sper. 27: 376–377, 1951.
24. Pansini, R. and Casaula, A. Sui rapporti tra alcolemia e metabolismo glicidico; l'alcolemia endogena nell'ipoglicemia insulinica nei soggetti normali. Boll. Soc. ital. Biol. sper. 27: 378–380, 1951.
25. Pansini, R. and Casaula, A. Studi sull'alcolemia endogena; l'alcolemia prima e dopo salasso. Boll. Soc. ital. Biol. sper. 27: 380–382, 1951.
26. Jokipii, S. G. Experimental studies on blood alcohol in healthy subjects and in some diseases. Ann. Med. exp. Biol. Fenn., Vol. 29, Suppl. 2. Helsinki; 1951.
27. Bücher, T. and Redetzki, H. Eine spezifische photometrische Bestimmung von Äthylalkohol auf fermentativem Wege. Klin. Wschr. 29: 615–616, 1951.
28. Cakl, J. Fysiologická hodnota alkoholu v krvi. (Physiological blood alcohol.) Čas. Lék. čes. 93: 689–691, 1954.
29. Zysk, J., Witkowski, J. and Kaleta, Z. Zastosowanie w lotnictwie próby Widmarka do ilosciowego oznaczania alkoholu we krwi. (The application in aviation of Widmark's method for the quantitative determination of alcohol in the blood.) Acta physiol. polon. 6: 421–440, 1955.
30. Dotzauer, G., Redetzki, H., Johannsmeier, K. and Bücher, T. Erprobung einer spezifischen Fermentmethode zur Mikrobestimmung von Äthylalkohol. Dtsch. Z. ges. gerichtl. Med. 41: 15–29, 1952.
31. Möllerström, J. Über die endogene Bildung und den Nachweis des Alkohols bei Störungen im Zwischenstoffwechsel. Ark. Kemi. Min. Geol. 18B (No. 6), 1944.
32. Alcohol in the blood. (Queries and Minor Notes.) J. Amer. med. Ass. 161: 669–670, 1956.
33. Paulus, W. and Mallach, H. J. Untersuchungen von Diabetikern nach Widmark und nach der ADH-Methode. Dtsch. med. Wschr. 79: 1045, 1954.
34. Pansini, R. and Mazzone, R. L'effetto del carico di glicoso sull'alcolemia arterovenosa endogena dell'uomo sano. Boll. Soc. ital. Biol. sper. 28: 1464–1466, 1952.
35. Mauro, V. L'alcoolemia e la glicemia in soggetti normali e in diabetici sotto posto ad affaticamento. Rass. Med. industr. 10: 712–724, 1939.
36. Gutschmidt, J. Spezifität der Blutalkoholbestimmung nach Widmark. Klin. Wschr. 18: 58–59, 1939.
37. Candela, N. La curva alcoolemica in gravidanza, parto e puerperio. Ann. Ostet. Ginec. 52: 325–363, 1930.
38. Russo, S. C. Alcolemia nelle gravidanze e puerperi complicati. Fisiol. e Med. 2: 473–491, 1931.
39. Pansini, R. and Mazzone, R. L'alcolemia artero-venosa endogena dopo carico insulinico nel diabete mellito umano. Boll. Soc. ital. Biol. sper. 28: 663–664, 1952.
40. Pansini, R. and Casaula, A. L'alcolemia artero-venosa endogena dopo carico insulinico nell'uomo sano. Boll. Soc. ital. Biol. sper. 28: 709–710, 1952.
41. Pansini, R. and Casaula, A. Rilievi conclusivi sul bioritmo dell'alcolemia endogena nelle 24 ore. Boll. Soc. ital. Biol. sper. 28: 710–712, 1952.
42. Pansini, R. and Notaristefano, M. Cocarbossilasi ed alcolemia endogena

artero-venosa nell'uomo sano ed epatopatico. Boll. Soc. ital. Biol. sper. 23: 1159–1160, 1952.

43. Pansini, R. and Notaristefano, M. Effetti di un carico di ATP sull'alcolemia artero-venosa endogena dell'uomo sano. Boll. Soc. ital. Biol. sper. 28: 1775–1777, 1952.
44. Pansini, R. and Casaula, A. Il bioritmo dell'alcoole nelle 24 ore. Minerva med., Torino 43: 216–218, 1952.
45. Leonardi, G. Iperalcolemia endogena in condizioni di anossia. Boll. Soc. ital. Biol. sper. 23: 477–480, 1947.
46. Stotz, E. A colorimetric determination of acetaldehyde in blood. J. biol. Chem. 148: 585–591, 1943.
47. Jacobsen, E. Is acetaldehyde an intermediary product in normal metabolism? Biochim. biophys. Acta. 4: 330–334, 1950.
48. Jacobsen, E. The metabolism of ethyl alcohol. Pharmacol. Rev. 4: 107–135, 1952.
49. Raby, K. Relation of blood acetaldehyde level to clinical symptoms in disulfiram–alcohol reaction. Quart. J. Stud. Alc. 15: 21–32, 1954.
50. Chundela, B. and Janák, J. Quantitative determinations of ethanol besides other volatile substances in blood and other body liquids by gas chromatography. J. forens. Med. 7: 153–161, 1960.
51. Lester, D. A biological approach to the etiology of alcoholism. Quart. J. Stud. Alc. 21: 701–703, 1960.
52. Schulman, M. P., Zurek, R. and Westerfeld, W. W. The pathway of alcohol metabolism. In: Himwich, H. E., ed. Alcoholism: Basic Aspects and Treatment; pp. 29-37. Washington, D.C.; American Association for the Advancement of Science (Publ. No. 47); 1957.