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THE DETERMINATION OF ETHYL ALCOHOL IN THE BLOOD AND TISSUES, ITS ABSORPTION AND DISTRIBUTION AND ITS EFFECT UPON SOME OF THE BLOOD CONSTITUENTS OF THE RAT

by

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INTRODUCTION

The effects of alcohol upon man have been studied and discussed for many years during which time many facts have been established. The purpose of these experiments was to find some of the factors influencing the rate of absorption of alcohol into the blood and tissues of the rat and to study its influence upon some of the normal blood constituents. It seemed desirable to have such information, using the rat as an experimental animal, inasmuch as the results so obtained might be of value when applied to man.

These experiments were started at about the time the use of chemical analysis of blood was being suggested as an aid in the diagnosis of intoxication in man. This means of diagnosis was supported by some who believed it to be useful as legal evidence, as well as of clinical importance. It was not practical to attempt to establish a relationship between blood alcoholic concentration and intoxication in the rat, since the symptoms of intoxication in the rat are less easily observed than in man.

In order to study the absorption of alcohol into the blood, it was necessary to develop a method of analysis by which an accurate estimation of the alcohol could be made using a small sample of blood. The method was then used to study alcohol absorption and the influence of mode of administration and the presence of other substances upon the rate of absorption. The influence of ingested alcohol upon some normal blood constituents was studied in order to de-

termine if the apparent toxic effects of alcohol could be correlated with changes in the concentration of any such constituents of the blood.

The distribution of alcohol in the body tissues was investigated in order to establish a relationship between the alcoholic concentration in various tissues to that of the blood at certain intervals of time after the administration of alcohol. The tissues analyzed were chosen to include as many types of tissues as possible.

Certain limitations arising from the use of the rat as an experimental animal were recognized in connection with this experiment. Individual variations were expected and found to exist as might be true in the case of all living matter. Each individual rat was subjected to as many different experiments as was practical in order to obtain as complete a picture of the conditions as possible. The possibility was recognized that certain experiments might render an individual rat unsatisfactory for further experimentation; hence a series of experiments to be performed on a given rat was selected so that no one experiment would invalidate the others.

The albino rat, Rattus norvegicus, was used in all of these experiments in which animal experimentation was necessary.

The experimental work of this thesis was planned to furnish some information pertaining to the following problems:

1. The development of an accurate method by which the alcoholic concentration of the blood may be determined using a small sample.

2. The determination of alcohol in tissues and its distribution in the different tissues.
3. To study the absorption of alcohol into the blood following its oral administration using both fasted and unfasted rats.
4. The effect of habituation upon the absorption of orally administered alcohol.
5. The absorption of alcohol into the blood following intraperitoneal injection of various amounts of alcohol.
6. The effect of the oral administration of some substances along with the alcohol upon the absorption of the latter into the blood.
7. The effect of alcohol upon growth and the consumption of feed.
8. The effect of ingested alcohol upon certain normal blood constituents.

HISTORICAL

The literature dealing with the effects of ethyl alcohol on the individual is very voluminous. There seems to be no complete review of the entire subject available at the present time, although some aspects of the subject have been reviewed in an able manner. Jellinek and Jolliffe (57) reviewed the literature of 1939 with regard to the effect of alcohol on the individual, thus eliminating the discussion of social and medico legal aspects of the problem. This review cites 272 references under the date line of 1939. In order to ascertain the facts concerning the problems of alcohol and to present these facts in the most convenient manner, the Research Council on Problems of Alcohol was organized in 1938. The reports of this council are now published quarterly (87). These reports and papers cited should be invaluable in the organization of research on alcoholic problems.

The Determination of Alcohol

In the past few years, the quantitative determination of alcohol in the body tissues, fluids and expired air has received the attention of many investigators. Widmark (100), in 1922, reported a method for the determination of ethyl alcohol in the blood. This method has received the support of and has been criticised by many investigators.

Widmark's method, however, has been the basis for many of the determinations reported since 1922.

In 1931, Nicloux (84) reported a method differing in some respects from Widmark's. Shortly after that, Heise (48) proposed a method for use in connection with medico legal evidence in intoxication. Two years later, in 1936, Abels (1) reported a micro modification of Heise's method. In both of these methods the alcohol was oxidized in a mixture of excess potassium dichromate and sulfuric acid. The color produced was compared with that produced by the oxidation of definite amounts of alcohol under the same conditions. In Heise's method the alcohol was removed from the sample by distillation from a mixture of picric acid and tartaric acid.

Abels' method differed from Heise's in the way the alcohol was removed from the sample. The sample was absorbed onto a roll of filter paper and suspended above the dichromate-sulfuric acid mixture in a special flask. The flasks containing the material were heated to vaporize the alcohol and cause its oxidation by the dichromate. Sheftel (91) proposed a similar method, except the excess dichromate was determined using a colorimeter. A special blue filter (#554 MacGregor Instrument Co., Needham, Mass.) was used to correct for the color of the reduced dichromate.

In most of the methods which have been reported for the estimation of alcohol in body fluids, the alcohol was removed from the sample by distillation in the presence of some non-volatile deproteinizing agent or by desiccation. The aqueous solution of alcohol

so obtained was then treated with a potassium dichromate-sulfuric acid mixture and after the oxidation of the alcohol was completed, the excess potassium dichromate was estimated.

Harger (42), in 1935, first reported a very convenient and accurate method by which the excess dichromate could be determined. The alcoholic content of the blood was determined by the distillation of the alcohol from blood filtrate and oxidation of the alcohol in an aliquot of the distillate by a definite amount of dichromate in sulfuric acid. The excess dichromate was determined by titration with a solution of methyl orange and ferrous sulfate in 50 per cent (by volume) sulfuric acid. This means of the determination of excess dichromate was used by Cavett (12), Levine and Bodansky (66), Johnston and Gibson (58) and Fish and Nelson (18) in their methods.

Cavett used a modification of Abels' method to remove the alcohol from the sample. The sample was placed in a glass cup which was then suspended in a glass-stoppered flask above a measured amount of dichromate and sulfuric acid. The alcohol was removed and the oxidation promoted by heat. Levine and Bodansky (66), in 1939, gave a review of the methods available at that time, which, according to them, were most useful in the clinical pathology laboratory because of their simplicity and accuracy. The techniques of Heise, Abels, Sheftel, Newman, Harger and Cavett were reviewed.

In a number of available methods, the excess dichromate remaining after the alcohol from a sample had been oxidized, was determined by iodine. The following reactions were the basis of these methods:

$$K_2Cr_2O_7 + 6KI + 7H_2SO_4 \longrightarrow 4K_2SO_4 + Cr_2(SO_4)_3 + 3I_2 + 7H_2O$$

$$Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$$

These reactions were utilized in the methods of Miller and Getchell (73), Newman (80), Rapin (86), Winnick (104) and Jungmickel (61), using an improvement in the methods of Widmark (100) and Stempel (93).

Other means of measuring excess dichromate were used. Some investigators added an excess of ferrous sulfate and then titrated the excess ferrous sulfate with potassium permanganate. Fleming and Stotz (21) and Gibson and Blotner (28) determined the amount of dichromate reduced by the alcohol by means of a photo electric colorimeter and Goubau (30), in one of his methods, used a spectrophotometric method, which depended upon the absorption of ultra violet light of 350 millimicrons wave length by dichromate but not by trivalent chrome salts.

Beeman (4), and Gettler and Freireich (26) removed the alcohol from the sample by steam distillation. The alcohol was oxidized to acetic acid by an excess of dichromate and the acetic acid so produced was distilled under diminished pressure and titrated with a standard base. A large amount of sample was necessary in this method.

A very accurate method was published in 1936 by Friedemann and Klaas (24). In this method the sample was treated with sodium tungstate and mercuric sulfate-sulfuric acid solutions. The alcohol was removed by distillation. A second distillation was used if the

sample contained aldehydes or ketones. This second distillation was made from a mixture of calcium hydroxide and mercuric sulfate. The alcohol so obtained was oxidized under carefully controlled conditions by means of alkaline potassium permanganate. The excess permanganate was determined by acidification with sulfuric acid and treatment with excess potassium iodide. The liberated iodine was titrated with standard sodium thiosulfate. This method was recommended by Hinsberg (50) for its specificity.

A specific, but very tedious method, was reported by Kluge (62), in 1939. In this method the alcohol was isolated as ethyl 3,5-dinitrobenzoate. The ester was saponified and the alcohol so obtained was then determined. This method required a large sample and considerable time, but was very specific and valuable for medico legal work. Docker (15) made use of the interferometer to determine alcohol in blood. An aerometric method has been devised by Harger, Bridwell and Raney (44).

A very good critical review of the various chemical methods by which alcohol may be determined in body fluids and tissues was given by Jetter (54), published in 1941. The use of chemical analysis for alcohol was reviewed by Seleznick (90), in 1938.

A number of methods have been proposed for the determination of alcohol in expired air. The estimation of the alcoholic content of expired air is employed as evidence of intoxication. Only a few methods will be mentioned here since they were not to be used in this investigation.

Haggard and Greenberg (34) reported a method in 1934 which was used to determine alcohol in air, blood and urine. The basis of this determination was the reaction of alcohol with iodine pentoxide. The following reactions were involved:

$$5C_2H_5OH + 6I_2O_5 \longrightarrow 10CO_2 + 15H_2O + 6I_2$$

$$C_2H_5OH + I_2O_5 \longrightarrow 2CO_2 + 2H_2O + 2HI.$$

The iodine and hydriotic acid were determined separately. Greenberg and Keator (31) reported a method in 1941 which was used for the indirect determination of blood alcohol. The alcohol in expired air reacted with iodine pentoxide as shown above. There is a constant relationship between the iodine produced and the amount of alcohol in the sample. The iodine was determined photoelectrically by means of the blue color produced with starch. The relationship between the alcoholic content of blood to that in expired air is 1300:1.

Jetter, Moore and Forrester (56) used magnesium perchlorate to determine the alcohol in expired air. These investigators claimed that the per cent of alcohol in the blood could be calculated from the mg. of alcohol and mg. of CO_2 in the same sample of expired air asfollows (55):

$$\frac{0.2 \times \text{mg. alcohol}}{\text{mg. } CO_2} = \text{per cent alcohol in the blood.}$$

Harger, Lamb and Hulpieu (47) reported a method by which the alcoholic concentration of the blood could be determined indirectly by measuring its concentration in expired air.

There has been a great deal of discussion of the individual merits and disadvantages of the various methods and their specificity. Swim, McCowley and Leake (94) claimed that they found values up to 150 mg. per cent alcohol in normal blood by chemical methods. This claim has not been substantiated by most other investigators. An editorial (96) published in 1940 refuted these claims of unspecificity. Heise (49), Harger (43), Jetter (53) and Bavis (2) almost simultaneously refuted the claims of Swim, et al.

Jellinek and Jolliffe (57) claimed that the available methods were all satisfactory for the estimation of blood alcohol associated with intoxication. They also concluded that the most specific methods must be used to determine the normal alcoholic concentration of blood.

It was well recognized that experimental errors may be encountered in connection with taking samples and their treatment. Schoen (89) published in 1940 a review of the possibility of such errors. Several investigators have found the method of Friedemann and Klaas (24) more specific than that of Widmark (100). Hinsberg and Breutel (51) and Hinsberg (50) have pointed out this advantage. Jetter (54), in his review, pointed out that most of the recent methods are accurate within $\pm$ 3.0 per cent if carefully used. He recommended Harger's (42) method for the estimation of excess dichromate when the latter is used to oxidize the alcohol.

Since it is to be expected that the effects of alcohol upon an individual will depend quite closely upon the concentration of the

alcohol in nervous tissue, especially the brain, there have been a number of methods proposed for the analysis of tissue for alcohol.

Harger (42) proposed a method for tissue analysis involving the removal of the alcohol by steam distillation. Jetter (54) modified Harger's method in such a way that the steam distillation has been eliminated. Cettler and Freireich (26) removed the alcohol by steam distillation from the tissues or body fluids and determined the alcohol in the distillate by oxidation to acetic acid with excess dichromate. The acetic acid so produced was distilled and titrated with standard alkali. This method gives a low degree of accuracy. The method described in this thesis was published by Fish and Nelson (19). This method was a technical improvement of that described by Harger (42).

The Absorption and Distribution of Alcohol

Ethyl alcohol is rapidly absorbed from the digestive tract and is distributed quite rapidly in the various tissues of the body. The absorption and distribution of alcohol in the body has been studied by a large number of investigators and the literature dealing with this subject is very extensive.

Mellanby (71), in 1919, reported a rather extensive study of the absorption into and disappearance of alcohol from the blood. He found that the maximum concentration in the blood was reached in from 0.5 to 2 hours following the oral administration of alcohol. He also pointed out that fat decreased the absorption of strong, but

not weak alcohol solutions. He found that milk was the most effective substance which he studied to delay the absorption of alcohol. Mellanby concluded that the rate of oxidation of alcohol in the body was constant regardless of the concentration present. He gave a rate of 0.148 grams per kilo per hour. Several later investigators (35), (17), (83), (52), however, found that the rate of oxidation of the alcohol diminished as the concentration of the alcohol decreased. Carpenter (8), in 1940, reviewed the literature pertaining to the metabolism of alcohol in a very useful manner. There are 166 references cited in this review. A more complete and detailed review of this topic was written by LeBreton (65), in 1936.

One of the earlier reports on the absorption of alcohol into the blood from the digestive tract was written in 1899 by Grehant (32). This investigator found that the alcoholic concentration in the blood, following the oral administration of a certain alcoholic beverage (Absinthe), reached a certain level at which it remained constant for some time. This so-called "Grehant Plateau" has been mentioned by quite a number of investigators since 1899. Some of Grehant's data are given in table 1.

TABLE 1

THE ALCOHOLIC CONCENTRATION OF BLOOD OF DOGS FOLLOWING THE ORAL ADMINISTRATION OF DEFINITE AMOUNTS OF ABSINTHE

Ml. per kilo administered	Concentration of alcohol grams per 100 ml. blood (Time in hours)					
	0.5	1.0	1.5	2.0	2.5	3.0
2	0.05	0.19	0.17	0.16	0.14	0.14
4	0.14	0.22	0.31	0.33	0.33	0.33
6	0.39	0.42	0.47	0.47	0.47	0.47

Miles (72), Dybing and Rasmussen (16), Carpenter (8), and Cori, Villiaume and Cori (14), pointed out the influence of the concentration of the alcohol solution on its absorption from the digestive tract. Cori, et al., concluded that the rate of absorption was roughly parallel to the amount given. Alcohol up to 20 per cent concentration had little effect on the rate of its absorption. However, alcohol is absorbed more slowly from a 40 per cent solution than from a 20 per cent solution. It was evident that the higher concentration of alcohol decreased the rate of evacuation of the stomach.

Haggard, Greenberg and Lolli (38), in a recent publication, have questioned the conclusions derived from many investigations. They pointed out the fact that the concentration of alcohol in the blood was the resultant of the following three factors: (a) the rate at which alcohol enters the body by absorption from the

alimentary tract; (b) the rate of diffusion of the alcohol between blood and tissues; and (c) the rate at which alcohol is lost by oxidation and elimination. They pointed out that on the basis of these three factors the blood alcoholic concentration can be used to study one of the above factors only if the other two are known and controlled. It is well known that absorption may be far from complete even after the blood alcoholic concentration is decreasing. On this basis, it is not possible to determine the rate of oxidation from the decreasing alcoholic concentration in the blood. These authors gave very conclusive evidence that absorption is not complete when the blood alcoholic concentration reaches a maximum. They showed that the amount of unabsorbed alcohol in both man and rats depended upon the amount of alcohol administered and the concentration of the solution used. In table 2 are given some of their data.

TABLE 2

TIME REQUIRED FOR COMPLETE ABSORPTION OF ALCOHOL FROM THE DIGESTIVE TRACT OF RATS

Alcohol administered grams per kilo	Concentration per cent	Time for complete absorption, (hours)
1.0	15	2
1.0	30	3
1.0	50	3
3.0	15	4
3.0	30	5
3.0	50	6

In addition, from this work and others reviewed by these authors, the following conclusions were deduced as to the absorption of alcohol from the digestive tract: (a) the alcohol is absorbed rapidly from the stomach for a short time after which absorption from the stomach is very slow; (b) further absorption depends upon the rate of evacuation of the stomach; (c) the absorption from the intestine is rapid and independent of the concentration of alcohol, or the presence of food material; (d) the presence of food material has a marked influence upon the absorption from the stomach; and (e) the rate of oxidation varies with the weight of the individual and the amount of alcohol ingested.

Haggard, Greenberg and Rakieten (39) pointed out that differences may occur in the shape of the blood alcohol-time curve by reason of the source of the blood sample. Some of their data are given in table 3 showing the concentration of alcohol in the blood of the same individual.The samples were taken from different sources. Rats were used in these experiments.

It is obvious that the source of the blood sample has a marked influence upon the apparent concentration of alcohol in the blood. The fact that the venous blood gives a lower value is due to absorption of the alcohol by the tissues. The brain probably absorbs alcohol more slowly than some of the other tissues; hence the concentration of alcohol in jugular blood is not so much lower than the arterial blood. The differences are so great that the source

TABLE 3

THE EFFECT OF THE SOURCE OF THE BLOOD SAMPLE ON THE ALCOHOL CONCENTRATION-TIME CURVE. TWO GRAMS PER KILO WERE ADMINISTERED TO RATS

Time Minutes	Mg. per cent alcohol Source of sample		
	Arterial	Jugular	Venous
10	115	60	35
20	150	113	75
30	175	155	115
40	185	173	145
50	190	185	170
60	190	187	180
70	185	183	180
80	183	182	176

of the sample should be considered when comparing the data of various investigators. Unfortunately, some of the earlier investigators did not state the source of the blood used.

Alcohol may be absorbed into the blood in other ways than through the digestive tract. Carpenter (8) reviewed the absorption of alcohol into the blood from the digestive tract, rectum, urinary bladder, skin and by inhalation. He concluded that the alcohol was rapidly absorbed in all cases except by way of the skin. A comprehensive study of the absorption of alcohol from the rectum of man was reported by Carpenter (9) in 1925. He reported that 61 grams of alcohol in a 10 per cent solution was completely absorbed in 5.5 hours. If 25 grams or less were administered, absorption was complete in one hour.

Absorption of alcohol by way of the lungs in fowls has been studied by Carpenter (10), in hens by Carpenter and Babcock (11), Loewy and Von der Heide (69), and earlier by Grehant and Quinquaud (33). All of these investigators found that alcohol was absorbed quite rapidly from the lungs. Carpenter (8) concluded that "all these investigations indicate that alcohol is rapidly absorbed when introduced into the animal body in various ways and that the main limiting factor is the death of the animal."

The effect of food and other material upon the absorption of alcohol from the digestive tract has been studied by many investigators. Hanzlik and Collins (41), in 1913, studied the absorption of alcohol from various parts of the digestive tracts of dogs and cats. They reported that the presence of such substances as bile and bile salts, soap, cholesterin, lecithin and olive oil, inhibit absorption as does injury to the intestinal mucosa. Mellanby (71) also studied the influence of some materials upon absorption and concluded that milk either administered with or before the alcohol, inhibited its absorption from the digestive tract.

Beazell and Ivy (3), in a review of the effect of alcohol on the digestive tract, concluded that milk is better than other food to diminish absorption of alcohol from the digestive tract.

Haggard, Greenberg and Cohen (37), working on man, found that it took nearly double the amount of alcohol given after a meal to give the same concentration of blood alcohol, as alcohol given before

a meal. Haggard and Greenberg (36) found that glycine retards the absorption of alcohol and keeps the blood alcoholic level lower. Widmark (103) and Southgate (92), working with man and other animals, found that protein and some amino acids slow the absorption of alcohol. Widmark considered it probable that some kind of a reaction takes place between the alcohol and the amino acids. Alanine inhibits the absorption of alcohol from the digestive tract, but according to Westerfield, Stotz and Berg (99), it increases the rate of oxidation of the alcohol.

Although no attempt was to be made in work reported in this thesis to determine the rate of oxidation of alcohol by the body, it would be well to briefly mention some of the work done on this problem.

Some of the earlier investigators, by measuring the decline of the alcoholic concentration of the blood, concluded that the rate of oxidation of the alcohol was constant and was independent of the concentration. Widmark (101), (108), working with man, evolved a formula by which the fate of the alcohol could be expressed mathematically. He assumed that both the absorption and distribution of alcohol were always complete in a short time. Mellanby (71) made the same assumptions and reported the rate of oxidation as 0.148 grams per kilo body weight per hour. Other investigators have made the same assumptions. Haggard, Greenberg and Lolli (38), working with man and rats, show very good evidence that absorption

and distribution of alcohol are not complete in a short time. They point out that Widmark's (102) equation is wrong, since the rate of oxidation of alcohol is not constant, but is proportional to the concentration of alcohol present. Their conclusions as to the rate of oxidation and elimination of alcohol in man are shown in table 4.

TABLE 4

THE RATE OF OXIDATION OF ETHYL ALCOHOL IN MAN

Weight (kilos)	Alcohol administered (grams)	Rate of oxidation and elimination (grams per hour)
47 - 64	22	4.2 - 5.1
65 - 79	22	5.2 - 6.3
80 -112	22	6.3 - 7.4
47 - 64	44	5.7 - 6.5
65 - 79	44	6.5 - 7.3
80 -112	44	6.9 - 8.4

If the elimination of alcohol is quite constant, (which is assumed if the blood alcoholic concentration is determined indirectly by measuring the concentration of alcohol in expired air and urine), the rate of oxidation increases with the weight of the individual and the amount of alcohol given. Carpenter (8) concluded that there is no way to predict the rate of the metabolism of alcohol.

The influence of various substances upon the rate of metabolism

of alcohol has been studied by a large number of investigators. It is probable that some of these overlooked other factors which may cause an apparent change in the rate of metabolism such as absorption and distribution rates. Clark, Morrissey, Fazekas and Welch (13) recognized the controversy over the question of a constant rate of metabolism, but apparently assumed it to be constant in their study of the role of insulin and the liver in alcohol metabolism. They concluded that the rate of oxidation of alcohol (or rate of disappearance from the blood) is a constant in dogs and cats. They found that glucose alone had no effect upon the rate of oxidation, but the rate was increased if insulin and glucose or insulin, glucose and sodium bicarbonate were administered with the alcohol. This is not in agreement with Mirsky and Nelson (74), (75), who concluded that the alcohol is oxidized mainly in the liver and that the rate of oxidation does not depend upon carbohydrate metabolism. Mirsky and Nelson found that the oxidation of alcohol is diminished by anything which impairs the function of the liver. The only point of disagreement between Clark et al and Mirsky and Nelson is the role of increased carbohydrate metabolism (glucose and insulin).

The question probably can be settled only by further experimentation. However, at the present time, a glucose-insulin treatment is used in the treatment of intoxication. This treatment was questioned by Lolli and Greenberg (70), since they have found that

insulin increased the rate at which the stomach is evacuated when insulin is administered following the oral administration of 25 per cent alcohol solutions. Tennent (95) reported that the injection of 3 grams of ethyl alcohol per kilo body weight lowered the liver glycogen and raised the blood sugar of unfasted rats. Fasted animals did not show these changes nor was 1 gram of alcohol per kilo body weight sufficient to cause such changes in unfasted rats.

Westerfeld, Stotz and Berg (99) claimed the blood alcohol disappeared at the rate of 8 mg. per cent per hour. They found that the administration of pyruvates increased the rate to 21.1 mg. per cent per hour. Alanine had a similar effect but there was a one hour lag in its effect.

The effect of habituation on the absorption and disappearance of alcohol in the blood has been studied by several investigators of whom the majority found no difference. Those investigators who were unable to show a difference between habituated and non-habituated individuals were Levy (67), working with rats, Newman (81), Newman and Lehman (82), working with rats, and Mirsky, Piker, Rosenbaum and Lederer (76), working with man. The latter inventigators believed that the difference in behavior, produced by a given amount of alcohol between habituated and non-habituated individuals, was due to an acquired tolerance in the central nervous system. They all agreed that the differences were due to a hyposensitivity of the

body cells in the habituated individuals. Opposed to this view were Pringsheim (85), who used rats, and Gettler and Freireich (25), who believed the habituated individual was able to oxidize the alcohol more rapidly. Gettler and Freireich believed the tissue/blood ratio was lower in habituated individuals due to a more efficient oxidation system in the tissues. The evidence supporting the views of Gettler and Freireich is rather meager. It is, therefore, generally believed that the difference between the reactions of habituated and non-habituated individuals is due to an acquired tissue tolerance toward alcohol. The subject was reviewed in 1936 by Bogen (6) and further discussed by Newman (81), in 1941, who concluded that differences were due to an acquired tissue tolerance.

The distribution of alcohol in the tissues following its introduction into the body has been studied quite extensively. Alcohol has been found present in many of the tissues of various animals even though no alcohol had been given to the animal. Gettler, Niederl and Benedetti-Pichler (27) isolated pure anhydrous alcohol from a number of normal tissues from man, dogs and pigs. Some of their results are given in table 5.

Harger and Goss (45) found alcohol in the blood, urine, brain and kidneys of dogs. Liljestrand (68) wrote a review of the literature in 1938 dealing with the distribution of alcohol in the

TABLE 5

THE AVERAGE ALCOHOLIC CONTENT OF NON-ALCOHOLIC TISSUES

Source of tissue	Tissue	Mg. per cent alcohol
Human	Brain	0.40
Human	Liver	2.56
Human	Blood	4.00
Dog	Brain	3.00
Dog	Liver	0.70
Dog	Blood	1.30
Pig	Brain	0.07

tissues. Carpenter (8) concluded that the small amount of alcohol present in normal tissues is of no significance in the study of alcohol metabolism. Such values are necessary only to establish a zero point in studies on metabolism of alcohol.

Upon the administration of alcohol, the tissues absorb it from the blood until equilibrium is attained. This distribution in the tissues was reviewed by Carpenter (8). The distribution of alcohol in a number of animals has been reported. Carpenter and Babcock (11) showed that alcohol could be absorbed from the air into the tissues of dead hens. Vollmering (98), Hansen (40) and Harger, Hulpieu and Lamb (46) studied the distribution of alcohol in the tissues of dogs. In the experiments of Harger et. al. the alcohol was given by oral administration and blood samples were taken from the saphenous vein. In some cases, the alcohol was injected intravenously. These investigators concluded that there was no change in the distribution ratios

over a period of time from ¼ to 12 hours. It was pointed out that there was a lag in the case of muscle tissue. From the conclusions as to the distribution of alcohol, the values found in table 6 were calculated.

TABLE 6

THE RATIOS OF THE CONCENTRATION OF ALCOHOL IN TISSUES OF DOGS IN MG. PER CENT TO THE CONCENTRATION IN THE BLOOD AFTER 2-3 HOURS

Tissue	Ratio
Brain	0.85
Liver	0.78
Muscle	0.78
Spinal Fluid	1.18

Newman and Lehman (82), who used rats, found that the ratio between the alcoholic concentration in the brain to that in the blood was 0.73 and remained constant between 0.5 to six hours. The alcohol was administered by intravenous injection. Jetter (54) found a similar ratio of 0.74. Eggleston (17) found the ratios between the concentration of alcohol in the tissues and the plasma to be quite consistent for the brain, lung, heart, spleen and kidney, all between 0.7 and 0.8. Fatty tissue has been shown by Vollmering (98) to absorb alcohol very slowly and to hold it tenaciously.

Liljestrand (68) stated in his review that if one takes into account the degree of concentration of alcohol in the plasma and the

water content of the various tissues, the concentration of alcohol in the tissues agrees with the laws of diffusion. Kunkele (64) and Harger, Hulpieu and Lamb (46) found that the alcoholic content of the tissues varied directly with the amount of liquid in the tissue.

A number of investigators have determined an average distribution ratio between the body tissues and the blood by which the total alcoholic content can be calculated provided absorption is complete. Haggard, Greenberg and Rakieten (39) found this ratio to be 0.62. Carpenter (10) found the ratio to be 0.60. Widmark (101) found that by the periodic determination of alcohol in the blood and the weight of the individual, the total alcohol could be calculated using the ratio of 0.68 for men and 0.55 for women. Bernhard and Goldberg (5) found slightly different averages, for men 0.70 and for women 0.59. The total alcohol may be calculated as follows:

Total alcohol = blood alcohol x ratio x body weight

Cottler and Freireich (26) found very little agreement between the alcoholic concentration in the brain and in the blood of autopsied patients. They found ratios of alcohol in tissues to alcohol in the blood to be between 1.51 and 0.22.

Most investigators have found rather close agreement in their experiments on the ratio between tissue and blood alcohol. However, some may appear in disagreement especially in the studies made during the first 1.5 hours, since during that time the source of the sample of blood has a marked influence on the apparent amount of alcohol present

in the blood. Hence, if the ratio between the tissue and venous blood is given, it will be much higher than the ratio between the tissue and arterial blood.

The Effect of Alcohol Upon Growth

The effect of alcohol upon growth would be a result of the opposing reactions produced by it in the body. There are scarcely more than traces of alcohol found in the body and Mitchell and Curzon (78) have concluded that there is not enough direct evidence to support the belief that alcohol is a normal metabolite. As stated by Mitchell (77), in 1935, there is a great deal of confusion regarding the availability of the energy from ethyl alcohol. Some investigators have found an increased metabolic rate produced by alcohol indicating that the energy liberated by the oxidation of alcohol is dissipated as heat (specific dynamic action). However, other investigators found no effect produced by alcohol on the basal metabolism, although there was produced a lowering of the respiratory quotient resulting from the oxidation of the alcohol. The energy thus produced would be utilized in physiological processes. Mitchell (77) found that the energy of ethyl alcohol was largely available for physiological purposes. He further demonstrated that the energy of ethyl alcohol was about three-fourths as available as that of sucrose; hence producing a greater specific dynamic effect than sucrose.

Mitchell also concluded that ethyl alcohol could promote growth and the new tissues so produced had a higher than normal fat content. Mitchell and Curzon (78) concluded that ethyl alcohol showed little specific dynamic action in man; however, for unknown reasons, its energy is not as well utilized as that of glucose. The energy of ethyl alcohol will spare that of carbohydrates and fats, but not proteins. It, however, may increase the digestibility of proteins. The limiting factor in the use of the energy of ethyl alcohol by the body seems to be the inability of tissues other than the liver to oxidize it. The liver does not respond to the demands of the other tissues for an increased supply of energy; hence muscular activity does not seem to increase the rate of alcohol metabolism. It appears, therefore, that ethyl alcohol will support growth very effectively within limits.

Richter and Campbell (88) recently investigated the concentration of alcohol solutions preferred by rats. They found that rats preferred 2.4 to 4.2 per cent by weight solutions of alcohol to distilled water. Water was preferred to those solutions above 6 per cent alcohol.

Some Pathological Conditions Associated With Alcoholism

Although no extensive study of pathological developments was to be made, it seems worth while to mention some of the more pertinent literature on the subject.

Wright (105) published a review of the general pathology and special complications associated with alcoholism. He concluded that alcohol is definitely associated with cirrhosis of the liver but no other pathological conditions could be directly traced to alcoholism. It is quite generally agreed that the continued ingestion of alcohol leads to fatty infiltration of the liver replacing considerable of the glycogen store. Even the injection of 3 grams of alcohol per kilo body weight caused a decrease of liver glycogen according to Tennent (95).

Burger (7) has reviewed the literature relative to the effect of alcohol on the kidney. He concluded that no kidney damage has been reported which conclusively proves any direct damage to the kidney resulting from the ingestion of alcohol. The arteriosclerotic kidney was a possible exception according to Burger.

It is now quite generally accepted that many of the so-called alcoholic diseases are developed from some or all of four effects of alcohol upon the individual: (1) the irritant action on the gastric mucosae, (2) the interference with the absorption and utilization of the vitamins, (3) the substitution of vitamin-free alcohol for vitamin containing food and (4) the increased vitamin requirement in consequence of the calories furnished by the alcohol. This subject was treated in a thorough manner by Jolliffe (59), in 1940.

Jolliffe and Wortis (60) reviewed the literature in 1941 in which discussion they concluded that the "toxic" effects of the continued use of alcohol were due to avitaminosis.

EXPERIMENTAL

Plan of Investigation

It seemed desirable to conduct a few experiments to determine the influence of ethyl alcohol upon the growth of rats and to find the effect of different concentrations of alcohol upon food and liquid consumption. this information being desirable before experimentation on the influence of ethyl alcohol upon various blood constituents.

A search of the literature for a method by which the alcoholic concentration of the blood could be determined revealed no method which could easily be applied to the study of the absorption of alcohol into the blood using rats as experimental animals, since the amount of sample required was too large or the time for analysis was too long. It was desirable to develop a method by which the concentration of ethyl alcohol could be determined using 0.1 ml. of blood or less.

With such a method available, the absorption of ethyl alcohol into the blood was to be studied in both habituated and non-habituated rats. There are many factors which influence the absorption of any substance into the blood. The influence of the mode of administration was to be studied using both oral and intraperitoneal administration of alcohol solutions. The absorption of alcohol from the

digestive tract is influenced by the presence of material in it when the alcohol is given. The influence of such substances as whole milk, cream, skim milk and glucose was to be studied by the administration of these substances with the alcohol. The absorption of alcohol from the digestive tract of fasted and unfasted rats was to be compared in this connection.

No mention was found in the literature of the effect of continued ingestion of alcohol upon such normal blood constituents as hemoglobin, fasting blood sugar level, non-protein nitrogen and uric acid. These effects were to be studied and some modifications of the existing analytical methods for the determination of non-protein nitrogen and uric acid in the blood were to be made.

The distribution of alcohol in the tissues has been reported in the literature. It seemed desirable to improve the methods by which the alcoholic content of the tissues could be determined and to obtain some more data as to the distribution of alcohol at certain times after the oral administration of a given amount of ethyl alcohol.

Methods and Materials

Rations.

The basal ration fed to the rats used in this study was composed of the following in parts by volume: ground hulled oats 4 parts, ground yellow corn 4 parts, ground wheat 1 part, alfalfa meal 1 part,

tankage 0.5 part, linseed meal 0.5 part, buttermilk powder 0.5 part. To each 100 pounds of the above mixture were added 0.35 pound of bone meal and 0.5 pound of sodium chloride. The ingredients were obtained from a dealer in Ames, Iowa.

The animals were given alcohol solutions or water ad libitum.

Chemicals.

The C. P. ethyl alcohol used was obtained from the U. S. Industrial Chemical Corporation. The alcohol solutions used for consumption by the animals were prepared from this commercial alcohol by mixing with distilled water. The concentrations of these solutions were checked by chemical analysis, as well as by their specific gravity.

Standard alcohol solutions were prepared by dilution of purified commercial absolute alcohol.

The sodium tungstate used in the analytical work was obtained from the Mallinckrodt Chemical Works and was free from molybdenum. The methyl orange was obtained from the Hartman-Leddon Company. All other chemicals were the C. P. products of the J. T. Baker Chemical Company.

Analytical methods.

Alcohol in blood. The method used in this work has been reported in the literature by Fish and Nelson (18) and was developed in order

to make it possible to determine the alcoholic concentration of the blood rapidly using a small sample. The method used was a modification of the methods of Friedemann and Klaas (24) and of Harger (42).

The sample, 0.1 ml. or 0.05 ml. of freely flowing or oxalated blood, was diluted and rinsed into a Kjeldahl type distilling flask of about 50 ml. capacity, using 10 ml. of distilled water. To this mixture 1 ml. of 10 per cent sodium tungstate solution and 1 ml. of mercuric sulfate-sulfuric acid, containing 100 grams of mercuric sulfate and 56 ml. of concentrated sulfuric acid per liter, were added in order. The mixture was shaken and distilled at such a rate that 5 ml. of distillate were collected in from 10-15 minutes. The distillation apparatus was made entirely of glass. The Kjeldahl type flask was fitted to a vertical condenser by means of a ground glass joint. (A rubber stopper may be used instead of the ground glass joint with no apparent effects on the accuracy of the determination). The distillate was collected in a hard glass (pyrex) test tube, 22 mm. x 175 mm., which was graduated to contain 5 ml.

One ml. of 0.0434 N potassium dichromate solution, equivalent to 0.50 mg. of ethyl alcohol, was then added and mixed. Five ml. of concentrated sulfuric acid was then added and the contents of the tube mixed carefully so as to avoid boiling. The heat of dilution of the sulfuric acid was sufficient to cause the dichromate to completely oxidize the alcohol to acetic acid within 10 minutes. After 10 minutes or longer, the reaction mixture was cooled to room tem-

perature and the excess potassium dichromate was determined by titration with the reducing solution described by Harger (42). The reducing solution was prepared by adding 15 ml. of a 0.1 per cent solution of methyl orange, dissolved in 0.025 N sodium hydroxide, to 30 ml. of 60 per cent sulfuric acid. One ml. of a ferrous sulfate solution, containing 20 grams of salt and 12 ml. of concentrated sulfuric acid per 100 ml. of solution, was then added. The titre of this mixture remains constant for two to three days but should be standardized at least every two days. The end point in this titration is the first permanent pink tinge.

The titrations were made using a 10 ml. micro-burette graduated to 0.02 ml. An air stirring device was used to facilitate titration. The device consisted of a three-hole rubber stopper fitted on the burette tip which should be long enough to extend at least 1 inch through the stopper. A glass tube was inserted in another hole, this tube reaching nearly to the bottom of the reaction tube and the tip was constricted to about 0.5 mm. inside diameter. The third carried a tube which just extended through the stopper and was attached to a water pump. When a suction was applied the air drawn through the mixture stirred it thoroughly. Air had no effect upon the titration values. The burette was connected with controllable air pressure in order to force a fine stream of the reducing fluid into the solution being titrated. See Fig. 1.

Since the concentrated sulfuric acid contained a small amount of reducing substances, it was necessary to make a determination using

5 ml. of distilled water instead of the sample distillate. The procedure was the same as that used for the sample distillate. The reducing solution was standardized by titrating a sample or the water blank to the end point. Then 1 ml. of the standard potassium dichromate was added and the solution again titrated to the same end point.

The calculation of the alcoholic concentration of the blood was as follows:

$$\frac{\text{Ml. water blank - tit. of sample}}{\text{Ml. reducing sol. equiv. to 1 ml. 0.0434 N } K_2Cr_2O_7} \times \frac{0.5 \times 100}{\text{Ml. samp.}} = \text{Mg. per cent alcohol}$$

In order to check the accuracy of the method, a large sample of blood was treated with 0.15 per cent of a 1:1 mixture of sodium fluoride and potassium oxalate. This blood was analyzed for alcohol and then a series of samples containing known amounts of alcohol was prepared. These samples were analyzed by this method and by the Friedemann and Klaas method. The results are shown in table 7, and show good agreement between the micro method and the actual amount present.

In order to test the preserving action of the sodium fluoride-potassium oxalate anticoagulent, another series of blood samples was prepared containing known amounts of alcohol. These samples were stored in a refrigerator at 1°C and were analyzed for alcohol at intervals of one week for three weeks, using the micro method. The data are shown in table 8.

TABLE 7

COMPARISON OF RECOVERY OF ADDED ALCOHOL USING MICRO METHOD AND METHOD OF FRIEDEMANN AND KLAAS

Sample	Concentration of alcohol in mg. per cent		
	Actual	Micro	Friedemann and Klaas
1	Normal	5.0	4.5
2	47.5	48.5	44.5
3	129.0	128.5	130.4
4	171.7	172.3	166.5
5	258.0	256.5	240.0
6	309.0	307.0	307.8

TABLE 8

THE EFFECT OF TIME UPON ALCOHOLIC CONTENT OF BLOOD

Sample	Concentration of alcohol in mg. per cent				
		Time			
	Actual	0	1 week	2 weeks	3 weeks
1	Normal	6.0	6.0	6.2	5.3
2	35.2	35.3	36.0	36.1	36.8
3	69.0	69.0	70.0	67.2	68.5
4	119.0	119.5	118.0	117.0	116.0
5	161.0	156.0	159.0	158.0	145.0
6	228.5	228.0	224.0	228.0	218.0
7	299.0	300.0	292.0	292.0	292.0
8	338.0	336.0	336.0	335.0	333.0
9	375.0	376.0	368.0	368.0	363.0
10	404.0	403.0	400.0	400.0	396.0

The samples were warmed to 20° C. before the samples to be analyzed were measured and then returned to the refrigerator.

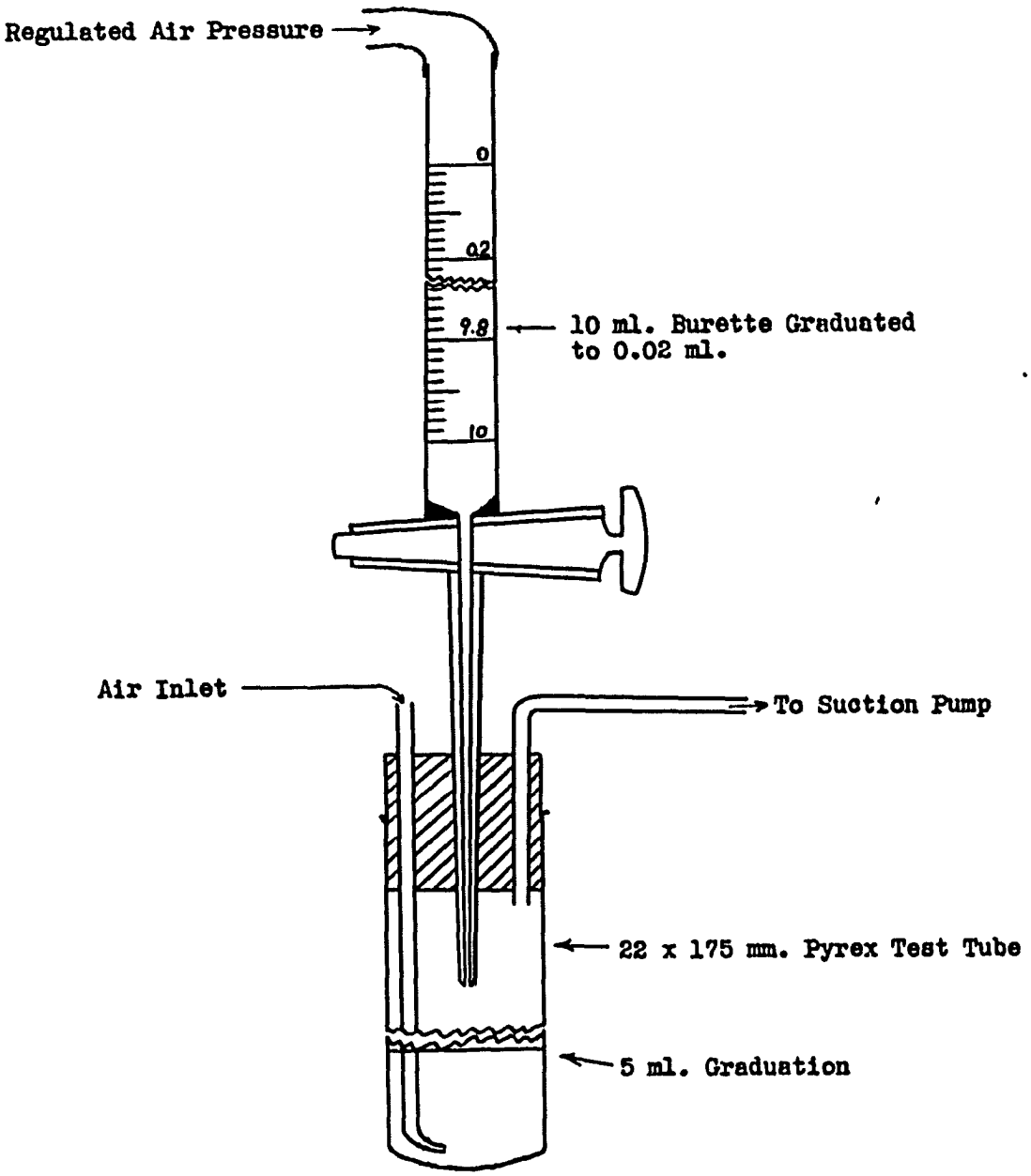

Fig. 1. Apparatus for Titration of Excess Dichromate Fittings for Air Stirring.

Preparation of standard alcohol solutions. A procedure was developed and reported in the literature (19) by which dilute solutions of ethyl alcohol of known concentration could be prepared.

A series of thin glass vials was prepared by drawing out a piece of glass tube to form a capillary of about 0.5 mm. inside diameter. A thin bulb was blown at one end of the capillary which would contain from 0.1 to 1.5 grams of alcohol when nearly filled. The capillary was then cut about 6 cm. from the bulb. The vials were dried and weighed. The vials were filled with pure ethyl alcohol by warming slightly, then placing the capillary in pure alcohol and the vials allowed to cool. To completely fill the vial, the small amount of alcohol in it was heated just to boiling and the capillary again inserted into the alcohol. Upon cooling, the vials nearly filled with alcohol. The open tip of the capillary was then sealed and the vials were again weighed. To prepare standard solutions of alcohol, a vial was broken under distilled water and diluted to volume.

The pure ethyl alcohol was prepared by treating some commercial absolute alcohol with slightly more than enough sodium necessary to react with all the water which might be present. After the reaction with sodium was complete, the alcohol was distilled using dry equipment. The middle one-third of the distillate was collected in a distillation flask fitted with a calcium chloride drying tube attached to the side arm. The distillate was preserved in a dry weighing bottle over concentrated sulfuric acid.

Alcohol in tissues. The method for the determination of alcohol in tissues, (19), was devised in order to simplify the procedures reported in the literature and at the same time minimize the chances of error.

The tissues were removed from the animal, which was killed by chloroform, as rapidly as possible and placed in weighed extraction flasks of 150 ml. capacity. The flasks were covered with small watch glasses and were cooled by small pieces of solid carbon dioxide placed in the flasks. As soon as the flasks were at room temperature, they were again weighed to obtain the weight of the tissues. The tissues were then covered with a solution of 10 per cent tartaric acid in half saturated picric acid and were stored in a refrigerator at 1° C. until the analysis could be finished.

The alcohol was removed from the tissue by means of a special apparatus so constructed that the sample could be subjected to steam distillation and hashed without being removed from the extraction flask. This apparatus was constructed in such a way that a three-hole rubber stopper which would fit the 150 ml. extraction flasks, carried a steam inlet, an outlet fitted with a trap leading to a condenser and the hashing knife. The steam inlet reached well into the flask so the steam was discharged below the surface of the liquid which covered the tissue. The hashing knife was all metal and consisted of a circular "saw-toothed" knife welded to the end of a solid rod. The construction was such that a mercury seal prevented

the loss of vapors even while running. The knife was turned by an electric motor. The outlet which led to the condenser was fitted with a trap to prevent material from being carried into the distillate mechanically. The details of the apparatus are shown in Fig. 2.

The steam distillate was collected in a volumetric flask of such a size that suitable aliquots could be taken which would contain less than 0.45 mg. of ethyl alcohol. If no more than 0.45 mg. of alcohol were expected, 10 ml. of distillate were collected directly in the distilling flask used for the analysis of blood. The aliquot used was diluted to 10 ml. with distilled water and then treated with 1 ml. each of 10 per cent sodium tungstate solution and mercuric sulfate-sulfuric acid solution added in order as in the analysis of blood. The mixture was distilled and 5 ml. of distillate collected. This second distillation was necessary, since the first distillate gave high values, probably due to the presence of steam volatile lipoidal material. The 5 ml. of distillate were treated as in the analysis of blood.

Calculation.

$$\frac{\text{Ml. water blank - ml. titration of sample}}{\text{Ml. reducing sol. equiv. to 1 ml. 0.0434 N } K_2Cr_2O_7} \times 0.5 = \text{Mg. alcohol in aliquot}$$

From this value the total alcoholic content and the concentration of alcohol in mg. per cent were calculated.

Several experiments were performed in order to check the accuracy of this method. A series of experiments was made in order to determine the recovery of alcohol added to the picric-tartaric acid solution.

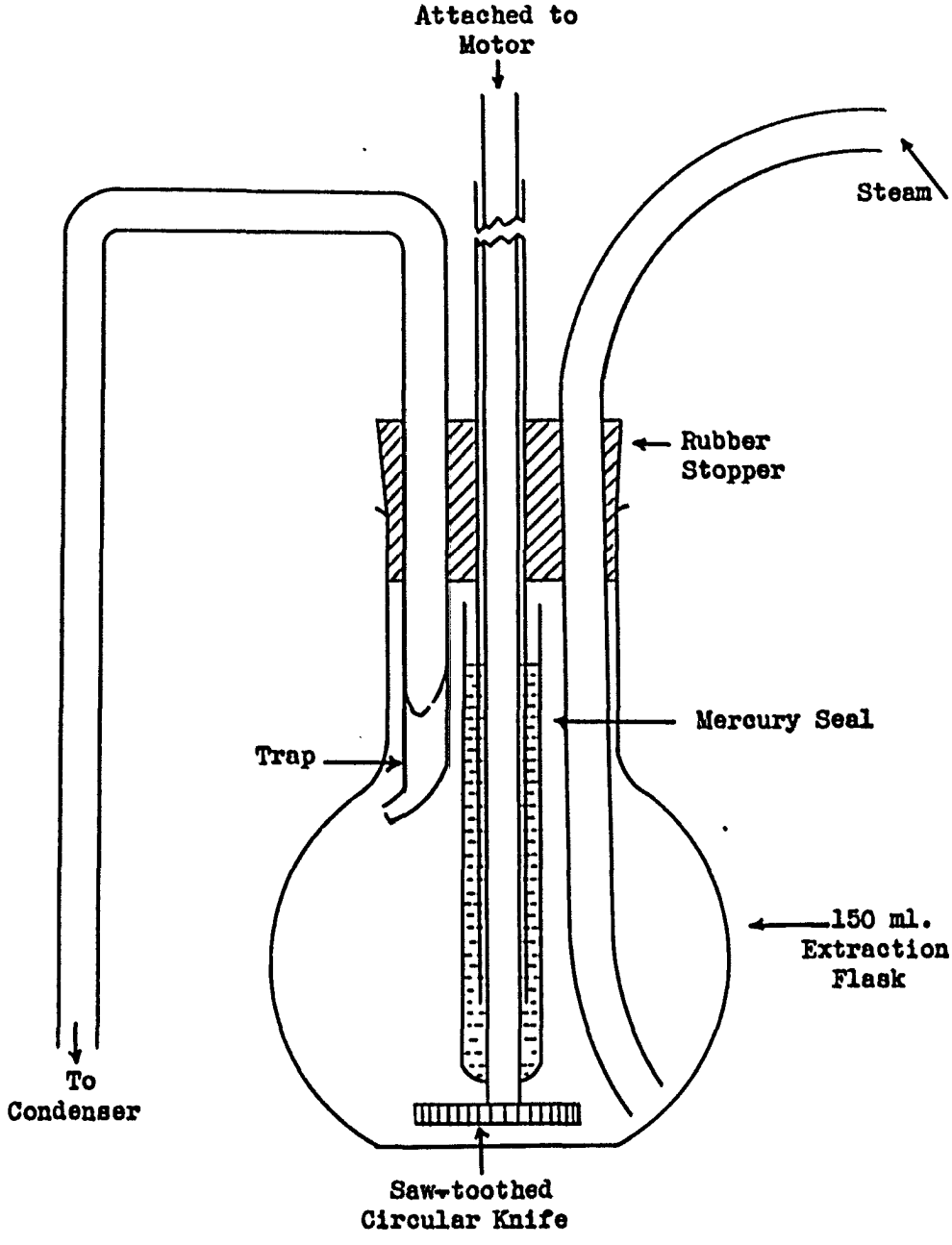

Fig. 2. Apparatus Used to Hash Tissues and to Remove the Alcohol by Steam.

Both distillations were carried out. The results of this series of experiments are given in table 9.

TABLE 9

THE RECOVERY OF KNOWN AMOUNTS OF ETHYL ALCOHOL

Sample	Mg. per cent ethyl alcohol		
	Actual	Found	% Recovery
1	3.06	3.10	101.3
2	3.06	3.08	100.5
3	1.83	1.85	101.1
4	1.83	1.84	100.5
5	0.91	0.90	98.8
6	0.91	0.91	100.0

In another series of experiments various tissues were removed and divided into two portions. One portion was analyzed as a blank and the other portion was treated with a definite amount of alcohol. The results of these experiments are shown in table 10.

Blood sugar determination. The Folin-Malmros (23) method for blood sugar was modified so that 0.05 ml. of blood could be used as a sample instead of the 0.1 ml. sample required in the original method. The quantity of dilute tungstic acid, used to precipitate blood proteins, was reduced from 10 ml. to 5 ml. This was measured into dry centrifuge tubes, having a total capacity of about 7 ml. and the deproteinization carried out as usual. After centrifugation, 4 ml. of supernatant solution, representing 0.0396 ml. of blood, were

TABLE 10

THE RECOVERY OF KNOWN AMOUNTS OF ETHYL ALCOHOL FROM TISSUES

Tissue	Wt. of sample (grams)	Mg. ethanol added	Mg. theoretical	Mg. found	% recovered
Liver	6.224	0	-	0.03	-
Liver	6.797	6.122	6.15	6.11	99.3
Spleen	0.502	0	-	0.02	-
Spleen	0.550	3.061	3.08	3.05	99.1
Kidneys	1.474	0	-	0.03	-
Kidneys	1.494	3.061	3.10	3.06	98.8
Muscle	2.508	0	-	0.04	-
Muscle	1.885	1.212	1.238	1.24	100.2
Testes	2.630	0	-	0.06	-
Testes	3.271	1.212	1.286	1.27	99.2
Brain	0.694	0	-	0.04	-
Brain	0.681	1.818	1.858	1.846	99.4

pipetted out for analysis. The remainder of the analysis was the same as the original method.

Calculation.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times (0.04) \times \frac{100}{0.0396} = \text{Mg. of glucose in 100 ml. blood}$$

The 0.04 represents the mg. of glucose in 4 ml. of the standard used for colorimetric comparison.

Hemoglobin. The hemoglobin concentration of the blood was determined in these experiments by the Newcomer method (79). In this method the blood sample was measured by means of a special dilution pipette and diluted to volume with 0.1 N hydrochloric acid.

The brown color developed was compared with a standard plate in the Newcomer hemoglobinometer. The instrument was graduated directly in per cent hemoglobin. The blood samples were taken from the tail of the animal or from the external saphenous vein. No differences could be found due to the source of the sample from a given animal.

The determination of uric acid in blood. The method used was a micro modification of Folin's (22) isolation method. Five ml. of tungstic acid solution were placed in a clean dry centrifuge tube. The tungstic acid was prepared by placing 20 ml. of 10 per cent sodium tungstate solution, free of molybdenum, in a 500 ml. volumetric flask and diluting to about 400 ml. with water. Twenty ml. of 2/3 N sulfuric acid were then added and the solution diluted to volume with water. The sample, 0.1 ml. of freely flowing blood, was measured with an accurate pipette and discharged into the tungstic acid. The pipette was rinsed by drawing the mixture of blood and tungstic acid into it several times. The contents of the centrifuge tube was mixed thoroughly and centrifuged after about 15 minutes. This treatment precipitated all of the blood proteins and gave a clear supernatant solution, 4 ml. of which were measured into a clean centrifuge tube. To this solution was added 1 ml. of acid silver solution, prepared according to Folin's directions, and the mixture was centrifuged at once. The supernatant solution was decanted without delay and the precipitate dissolved in 3 ml. of a urea-cyanide solution prepared according to Folin's directions. The color was developed in the unknown by the addition of 1 ml. of Folin's color reagent and 1 ml. of

water. The standard was prepared by treating 1 ml. of standard uric acid solution, containing 0.002 mg. of uric acid with 3 ml. of urea-cyanide solution and 1 ml. of color reagent. The color reagent was added to both the standard and the unknown at as nearly the same time as possible and after 20 minutes they were compared in a micro colorimeter.

Calculation.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.002 \times \frac{100}{0.1 \times \frac{4}{5.1}} = \text{mg. per cent uric acid}$$

Several experiments were performed in order to investigate some factors which might influence the values obtained by this method. The influence of alcohol in the blood was determined and no effects were shown. Some data are shown in table 11.

TABLE 11

THE EFFECT OF BLOOD ALCOHOL UPON APPARENT URIC ACID CONCENTRATION AS DETERMINED BY THE MICRO METHOD

Sample	Mg. per cent alcohol	Mg. per cent uric acid
1	5	1.88
2	5	1.84
3	150	1.86
4	200	1.87
5	306	1.83
6	350	1.86

The effects of storage of the blood in a refrigerator at 1° C.

were studied. Lithium oxalate was used as an anti-coagulent in one experiment and a mixture of lithium oxalate and lithium fluoride in another. The results are shown in table 12.

TABLE 12

THE EFFECT OF PRESERVATION OF BLOOD SAMPLES UPON THE APPARENT URIC ACID CONTENT USING THE MICRO METHOD

Time (hours)	Mg. per cent uric acid	
	Lithium oxalate	Lithium oxalate plus lithium fluoride
0	1.85	1.63
24	2.91	-
48	5.00	5.13
120	6.90	-

In some instances in which lithium oxalate was used as an anti-coagulent, very low normal uric acid values were obtained. It may have been that an excess of lithium oxalate was responsible and should be avoided.

Normal uric acid values obtained by this method on 25 different samples of rats' blood, using freely flowing blood, ranged from 1.2 to 2.1 mg. per cent with the average about 1.7 mg. per cent.

A few studies were made on the recovery of known amounts of uric acid added to a blood sample which had been analyzed for uric acid. Table 13 shows the results of one of these studies.

TABLE 13

THE RECOVERY OF KNOWN AMOUNTS OF URIC ACID ADDED TO BLOOD USING THE MICRO METHOD

Ml. blood sample	Mg. uric acid added	Mg. per cent theoretical	Mg. per cent found
0.1	0	-	1.20
0.1	0	-	1.22
0.1	0.001	2.21	2.15
0.1	0.001	2.21	2.18
0.1	0.002	3.21	3.20
0.1	0.002	3.21	3.22
0.1	0.003	4.21	4.25
0.1	0.003	4.21	4.23

Determination of non-protein nitrogen. The non-protein nitrogen was determined in the protein free blood filtrate obtained by adding 0.1 ml. of freely flowing blood to 5 ml. of tungstic acid prepared as described in the determination of uric acid on page 43 of this thesis. Four ml. of the supernatant fluid was measured into a pyrex digestion tube 200 mm. x 25 mm. which was graduated to contain 35 ml. One ml. of ammonia-free sulfuric acid, diluted with an equal volume of water, was added. The mixture was heated, using a micro-burner, until the water was evaporated and dense fumes of sulfuric acid filled the tube. A glass bead was used to prevent bumping during the heating. The mixture was allowed to cool slightly and then one drop of 30 per cent hydrogen peroxide was allowed to drop directly into the solution. Boiling was then con-

tinued for five minutes. After this treatment a clear solution resulted, except in some cases in which tungstic oxide was precipitated. This precipitate dissolved upon the later addition of Nessler's solution. After the solution was cool, 5 ml. of distilled water were added and the solution again cooled. After the solution was cooled, 15 ml. of a modified Nessler's (97) solution were added and the mixture diluted to 35 ml. with distilled water. The color produced was compared in a colorimeter with that produced by the same treatment of a standard containing 0.03 mg. of nitrogen as ammonium sulfate dissolved in 1 ml. of water. The standard used was prepared by dissolving 0.1415 grams of purified ammonium sulfate in distilled water and diluted to 1 liter.

Calculation.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.03 \times \frac{100}{0.1 \times 4/5.1} = \text{Mg. of non-protein nitrogen per 100 ml. of blood}$$

This method was checked against Koch's and McMeekin's (63) method and found to agree very well. It was found necessary to run blank determinations on the reagents used in order to correct for the small amounts of ammonia occasionally found.

Administration of substances by stomach tube.

The stomach tubes used in this study were made from size 8 French style rubber catheters. The catheters were cut at lengths of about 5 inches which were more than long enough to reach the stomach

of the largest rats used. One end of the stomach tube was securely fastened to the hub of a large hypodermic needle from which the needle had been removed.

The animals were subjected to a light chloroform anesthesia so the stomach tube could be inserted without resistance from the animal. The stomach tube was inserted into the esophagus and was then pushed on to the stomach with a rotating motion. The solution to be administered was forced slowly into the stomach by means of a 5 ml. Becton-Dickinson syringe. The operation required about one minute. The animals were recovering from the anesthesia before the stomach tube could be removed.

Administration of substances by intraperitoneal injection.

In the injection experiments a 5 ml. Becton-Dickinson syringe, graduated to 0.1 ml. was used with a 26 guage needle. The solutions used for this and for oral administration were of such concentrations that the desired amount could be obtained by the use of 1 ml. per 100 grams body weight. The injections were made into the peritoneal cavity by holding the animal on its back in the palm of the left hand with the fingers firmly restraining the hind legs. The thumb of the left hand held the middle portion of the animal's body and the head was held between the left arm and the body of the operator. The needle was inserted into the lower right side of the peritoneal cavity and the injection made as quickly as possible. The instruments were as clean and sterile as practical. No infections developed in the animals so treated.

Techniques of taking blood samples.

Blood samples of 0.05 ml. and 0.10 ml. were taken from the external saphenous vein, while larger samples were taken from the heart. When samples were taken from the external saphenous vein the animals were held, back down, between the left arm and the body of the operator. The hind leg was held firmly between the thumb and index finger of the left hand. The second and third fingers of the left hand were used to support the leg of the animal from below. The hair was clipped from the inner surface of the hind leg exposing the skin directly covering the external saphenous vein, the outline of which was easily visible. The vein was punctured by a lancet and the sample measured with an accurate pipette. Care was taken not to sever the vein while puncturing it. The most satisfactory type of lancet used was shaped at the tip similar to a nail. This construction made it easier to puncture the skin and vein without extensive damage to the vein.

Blood samples were taken from the heart while the animals were under chloroform anesthesia. The anesthesia was very important in these operations. It had to be deep enough to prevent any resistance on the part of the animal, but not so profound as to cause death. When the animal was correctly anesthetized, it was placed on its back and held in position by the left hand. The needle was inserted horizontally into the heart with the right hand. The blood was withdrawn by means

of a syringe and placed into an oxalated tube.

Care of animals.

Feeding. The animals had feed available at all times except in those experiments which involved fasting periods. The feed was supplied in metal cups placed inside a larger container in order to prevent scattering during feeding experiments.

Drink. In many of the experiments alcohol solutions were supplied instead of water. The alcohol solutions were made available to the rats in carefully constructed watering bottles so as to avoid loss through leakage. Rats on fasting experiments received water to drink.

Housing. The animals were kept in galvanized iron wire cages large enough to accommodate six adult rats. The rats were kept on galvanized iron wire screens. The cages were cleaned thoroughly at least once each week. The rats received daily observation and care throughout the duration of the experiments.

The Distribution of Ethyl Alcohol in the Tissues

Purpose.

These experiments were performed in order to obtain the concentrations of alcohol in various tissues at different times following the oral administration of ethyl alcohol. It seemed desirable to compare

the alcoholic concentration of each of the tissues with that of the blood.

Procedure.

The rats used in these experiments were between 150 and 200 days of age and varied in weight between 250 and 400 grams. The rats were fasted for 24 hours before the administration of alcohol solution. Some of the rats received no alcohol and their tissues were analyzed for the normal alcohol concentration of the tissues. The distribution of alcohol was determined after the oral administration of 2.5 grams of alcohol per kilo body weight. After a definite time a blood sample was taken and the animal killed with chloroform. The tissues were removed and analyzed as described on page 38 of this thesis. All of the rats used were normal animals in all respects and had not been subjected to other experiments.

Results and interpretations.

The normal alcoholic concentration of various tissues were determined in six normal rats. The results of these determinations are shown in table 14. All of these rats were males weighing between 265 and 384 grams. The average values of the six normal rats are given and the average tissue/blood ratios are also included. The alcoholic concentration of the tissues are expressed as mg. of alcohol per 100 grams of tissue.

A series of six rats were given 2.5 grams of ethyl alcohol per kilo body weight by stomach tube, after having fasted 24 hours. These rats were killed 30 minutes after receiving the alcohol and the blood and tissues were analyzed for alcohol. The results of these experiments are shown in table 15.

The average alcohol concentration of the tissues and the average tissue/blood concentration ratios are given. The tissue/blood ratios probably had the greatest meaning, since the ratios are less affected by different absorption rates of the individual rats.

In another series of similar experiments, the fasted rats were given 2.5 grams of alcohol per kilo body weight and killed two hours after the oral administration of the alcohol. The results of these experiments are shown in table 16. Again the tissue/blood ratios of alcoholic concentrations are given.

On the basis of experiments reported in the literature (101), (46), (82), it was expected that the tissue/blood alcoholic concentration ratios would remain quite constant until the alcohol had been metabolized or eliminated and only the normal amounts remained. In order to investigate this matter further, another series of experiments was made on rats fasted 24 hours. The rats were given 2.5 grams of ethyl alcohol per kilo body weight by means of a stomach tube. After a period of four hours, the tissues were removed and analyzed as in the preceding experiments. The results of these experiments are shown in table 17. Table 18 shows the average tissue/blood alcoholic concentration ratios at various times studied.

Rat	Liver		Heart		Brain		Spleen		Kidneys	
	Conc.	T/B*	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B
WR	2.30	0.51	2.40	0.53	4.30	0.95	4.50	1.00	1.20	0.27
BW	1.20	0.24	1.75	0.35	3.40	0.68	5.90	1.20	1.20	0.24
B	0.70	0.14	2.60	0.53	1.90	0.39	4.00	0.82	1.20	0.24
W_L	0.50	0.11	3.30	0.73	2.00	0.44	4.40	0.98	2.20	0.49
W	0.70	0.13	3.90	0.76	3.20	0.63	2.60	0.51	1.00	0.19
W_{HL}	1.10	0.18	2.40	0.40	1.70	0.28	4.10	0.68	2.65	0.44
Mean (Normal)	1.0	0.22	2.7	0.69	2.8	0.56	4.3	0.86	1.6	0.31

*The ratio T/B is the mg. per cent alcohol in the tissue divided by the mg. per cent

**The contents of the stomach and small intestine were washed out with distilled water

Table 14. The Normal Alcoholic Content of the Tissues of Fasted Rats

					Mg. per cent alcohol								
	Kidneys		Pancreas		Stomach** (washed)		Small intestine** (washed)		Muscle		Bone		
an T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc
1.00	1.20	0.27	0.60	0.13	3.40	0.75	1.30	0.29	0.30	0.06	-	-	0.8
1.20	1.20	0.24	2.50	0.50	4.00	0.80	2.00	0.40	0.40	0.08	1.50	0.30	0.5
0.82	1.20	0.24	0.70	0.14	3.20	0.65	1.60	0.33	0.40	0.08	0.50	0.10	0.6
0.98	2.20	0.49	1.60	0.36	6.30	1.40	2.20	0.49	0.80	0.18	1.20	0.27	0.9
0.51	1.00	0.19	2.10	0.41	3.40	0.67	1.60	0.31	2.30	0.45	-	-	1.4
0.68	2.65	0.44	2.90	0.48	6.10	1.02	1.00	0.17	2.60	0.43	1.00	0.17	1.2
0.86	1.6	0.31	1.7	0.34	4.4	0.88	1.6	0.33	1.1	0.21	1.0	0.21	0.9

by the mg. per cent alcohol in the blood.

with distilled water and the organs pressed dry in a clean towel.

it of the Tissues of Fasted Rats

alcohol ie**	Muscle		Bone		Testes		Lungs		Blood
3	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.
)	0.30	0.06	-	-	0.80	0.16	1.20	0.27	4.50
)	0.40	0.08	1.50	0.30	0.50	0.10	-	-	5.00
5	0.40	0.08	0.50	0.10	0.60	0.12	1.70	0.35	4.90
)	0.80	0.18	1.20	0.27	0.90	0.20	1.60	0.35	4.50
L	2.30	0.45	-	-	1.40	0.27	3.60	0.51	5.10
)	2.60	0.43	1.00	0.17	1.20	0.20	1.90	0.32	6.00
5	1.1	0.21	1.0	0.21	0.9	0.18	1.8	0.36	5.00

ol.

Table 15. The Concentration of Alcohol in the Tissues of Rats 30 Minutes After Re ceivi

Rat	Liver		Heart		Brain		Spleen		Kidneys		
	Conc.	T/B*	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/	B
B_{30}	48.2	0.68	32.4	0.46	10.7	0.15	49.3	0.70	42.2	0.	60
W_{30}	149.0	0.60	68.8	0.30	58.8	0.24	152.5	0.62	138.2	0.	55
W'_{30}	71.3	0.71	24.0	0.24	13.7	0.14	69.7	0.70	68.3	0.	68
W''_{30}	73.2	0.75	48.5	0.50	15.4	0.16	76.7	0.80	60.0	0.	62
BW_{30}	102.5	0.75	67.3	0.50	46.9	0.35	100.5	0.75	95.6	0.	72
W_{R30}	94.5	0.73	68.0	0.53	30.7	0.24	95.7	0.75	95.4	0.	74
Mean	89.8	0.70	51.5	0.42	35.2	0.21	90.7	0.72	83.3	0.	65

*The ratio T/B is the mg. per cent alcohol in the tissue divided by the mg. per cent

**The contents of the stomach and small intestine were washed out with distilled wate

ts 30 Minutes After Re ceiving 2.5 Grams of Ethyl Alcohol Per Kilo Body Weight. The Rats Were Previ

Spleen		Kidneys		Mg. per cent alcohol									
				Pancreas		Stomach** (washed)		Small intestine** (washed)		Muscle		Bone	
c. :	T/B	Conc. :	T/	B Conc. :	T/B	Conc. :	T/B	Conc. :	T/B	Conc. :	T/B	Conc. :	T
3	0.70	42.2	0.	60 38.6	0.55	2,360	33.60	233.0	3.30	28.4	0.40	15.1	0.
5	0.68	138.2	0.	55 88.2	0.36	1,950	7.90	89.2	0.36	53.8	0.22	42.2	0.
.7	0.70	68.3	0.	68 35.2	0.35	1,840	18.40	87.8	0.87	43.2	0.45	21.6	0
.7	0.80	60.0	0.	62 46.7	0.49	1,828	19.00	115.0	1.20	48.3	0.50	11.2	0
.5	0.75	95.6	0.	72 48.1	0.36	2,385	17.85	77.7	0.56	42.6	0.31	26.4	0
.7	0.75	95.4	0.	74 46.8	0.37	2,204	17.20	210.6	1.65	45.7	0.36	31.1	0
.7	0.72	83.3	0.	65 50.6	0.41	2,094	18.90	135.5	1.33	43.5	0.37	24.3	0

ivided by the mg. per cent alcohol in the blood.

hed out with distilled water and the organs pressed dry in a clean towel.

Weight. The Rats Were Previously Fasted 24 hours. The Alcohol was Administered by Stomach Tube.

ent alcohol

Muscle		Bone		Testes		Lungs		Blood
Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.
28.4	0.40	13.1	0.19	28.9	0.	41.5	0.59	70.2
53.2	0.82	42.2	0.17	65.0	0.	67.4	0.27	247.0
43.2	0.43	21.8	0.22	54.2	0.	70.7	0.70	100.5
48.3	0.50	11.2	0.12	60.8	0.	41.4	0.43	96.1
42.6	0.31	26.4	0.19	66.0	0.	99.4	0.72	137.0
45.7	0.36	31.1	0.24	63.1	0.	98.3	0.77	128.0
43.5	0.37	24.3	0.19	56.2	0.	69.8	0.58	129.8

1.

Table 16. The Concentration of Alcohol in the Tissues of Rats Two Hours After Receiv ing 2

Rat	Liver		Heart		Brain		Spleen		Kidneys		Pa
	Conc.	T/B*	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.
W_2	31.4	0.80	24.1	0.61	21.1	0.53	38.1	0.96	32.4	0.82	28.3
B_2	50.4	0.62	38.3	0.47	60.9	0.74	68.0	0.83	61.2	0.75	61.4
W'_2	277.0	0.88	158.0	0.50	235.0	0.75	239.0	0.76	241.0	0.76	153.0
WL_2	88.8	0.70	57.5	0.46	81.7	0.65	97.3	0.78	93.7	0.75	40.2
BR_2	165.0	0.75	110.0	0.50	154.0	0.70	184.5	0.84	169.5	0.77	101.0
BW_2	144.2	0.72	103.5	0.52	136.4	0.68	146.0	0.73	148.2	0.74	100.6
Mean	126.1	0.74	82.0	0.51	115.0	0.67	128.8	0.82	125.0	0.76	72.4

*The ratio T/B is the mg. per cent alcohol in the tissues divided by the mg. per c ent

**The contents of the stomach and small intestine were washed out with distilled wa ter

) Hours After Receiv ing 2.5 Grams of Ethyl Alcohol Per Kilo Body Weight. The Rats Were Previously F

			Mg. per cent alcohol										
	Kidneys		Pancreas		Stomach** (washed)		Small intestine** (washed)		Muscle		Bone		Te
T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.
).96	32.4	0.82	28.3	0.72	2,190	55.5	369.0	9.36	32.4	0.82	21.4	0.54	19.2
).83	61.8	0.75	61.4	0.75	1,880	22.9	166.5	2.00	61.8	0.75	25.6	0.31	46.0
).76	241.0	0.76	153.0	0.49	399	1.27	125.0	0.40	245.0	0.78	79.0	0.25	204.0
).78	93.7	0.75	40.2	0.32	1,051	8.40	56.5	0.45	65.7	0.52	57.8	0.46	69.2
).84	169.5	0.77	101.0	0.46	880	4.00	136.0	0.62	176.0	0.80	74.8	0.34	134.2
).73	148.2	0.74	100.6	0.50	1,100	5.50	116.0	0.58	142.3	0.71	60.5	0.30	106.0
).82	125.0	0.76	72.4	0.54	1,250	16.26	161.5	2.23	120.5	0.73	53.2	0.37	96.4

)d by the mg. per c ent alcohol in the blood.

; with distilled wa ter and the organs pressed dry in a clean towel.

Weight. The Rats Were Previously Fasted 24 Hou rs. The Alcohol Was Administered by Stomach Tube.

per cent alcohol

Muscle		Bone		Testes			Lungs		Blood
Conc.	T/B	Conc.	T/B	Conc.	T/B	Co	Conc.	T/B	Conc.
32.4	0.82	21.4	0.54	19.2	0.49	39	.7	1.00	39.5
61.8	0.75	25.6	0.31	46.0	0.56	81	.0	0.99	82.0
245.0	0.78	79.0	0.25	204.0	0.65	280	.0	0.89	315.0
65.7	0.52	57.8	0.46	69.2	0.55	98	.4	0.78	125.5
176.0	0.80	74.8	0.34	134.2	0.61	200	.0	0.91	220.0
142.3	0.71	60.5	0.30	106.0	0.53	176	.0	0.88	200.0
120.5	0.73	53.2	0.37	96.4	0.56	146	.0	0.91	153.7

wel.

Table 17. The Concentration of Alcohol in the Tissues of Rats Four Hours After Re cei

Rat	Liver		Heart		Brain		Spleen		Kidneys		
	Conc.	T/B*	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T	/B
W_4	85.2	0.77	88.2	0.80	92.5	0.84	85.6	0.78	84.4	0.	77
W_{L4}***	56.8	0.60	56.7	0.60	28.2	0.30	80.7	0.85	69.0	0.	73
W'_4	95.7	0.59	118.3	0.73	87.3	0.54	125.0	0.77	124.5	0.	77
W_{R4}	92.7	0.63	103.0	0.70	88.3	0.60	117.5	0.80	115.0	0.	78
B_4	87.8	0.66	101.0	0.76	77.2	0.58	108.0	0.81	100.0	0.	75
B^W_4	59.8	0.61	65.6	0.67	55.0	0.56	74.5	0.76	68.6	0.	69
Mean	79.7	0.64	88.8	0.71	71.4	0.57	98.5	0.79	93.6	0.	75

*The ratio T/B is the mg. per cent alcohol in the tissues divided by the mg. pe r ce

**The contents of the stomach and small intestine were washed out with distilled wate

***Food material in stomach when killed.

ur Hours After Re ceiving 2.5 Grams of Ethyl Alcohol Per Kilo Body Weight. The Rats Were Previously

							Mg. per cent alcohol									
	Kidneys			Pancreas		Stomach** (washed)		Small intestine** (washed)		Muscle		Bone				
T/B	Conc.	T	/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Conc.	T/B	Co
0.78	84.4	0.	77	44.8	0.41	892	8.10	103.5	0.94	61.3	0.56	31.2	0.28	54
0.85	69.0	0.	75	49.5	0.52	750	7.96	163.0	1.72	67.6	0.73	32.4	0.33	50
0.77	124.5	0.	77	68.0	0.42	2,220	13.75	210.0	1.30	110.0	0.68	31.4	0.20	86
0.80	115.0	0.	78	64.7	0.44	1,175	8.00	220.0	1.50	109.0	0.74	36.8	0.25	75
0.81	100.0	0.	75	50.5	0.38	1,112	8.42	215.0	1.62	91.8	0.69	28.0	0.21	64
0.76	68.6	0.	69	39.2	0.40	892	9.10	176.0	1.80	67.6	0.70	25.5	0.26	40
0.79	93.6	0.	75	52.8	0.43	1,178	9.22	181.2	1.48	84.5	0.68	31.0	0.25	62

ided by the mg. pe r cent alcohol in the blood.

out with distilled water and the organs pressed dry in a clean towel.

ght. The Rats Were Previously Fasted 24 Hours. The Alcohol Was Administered by Stomach Tube.

ohol

Muscle		Bone		Testes		Lungs		Blood
nc. :	T/B	Conc. :	T/B	Conc. :	T/B	Conc. :	T/B	Conc.
.3	0.56	31.2	0.28	54.2	0.49	95.5	0.85	110.0
.6	0.73	32.4	0.33	50.2	0.53	74.0	0.78	94.5
.0	0.68	31.4	0.20	88.2	0.54	130.0	0.80	162.0
.0	0.74	36.8	0.25	75.0	0.51	112.0	0.76	147.0
.8	0.69	28.0	0.21	64.0	0.48	106.0	0.82	133.0
.6	0.70	25.5	0.26	46.0	0.47	80.4	0.82	98.0
.5	0.68	31.0	0.25	62.9	0.50	99.3	0.80	124.1

Table 18. The Average Tissue to Blood Alcohol Concentration Ratios, at the Various Times Studied, Following the Oral Administration of 2.5 Grams of Alcohol Per Kilo Body Weight to Fasted Rats.

	T/B ratios at time in minutes			
Tissues	0	30	120	240
Liver	0.22	0.70	0.74	0.64
Heart	0.69	0.42	0.51	0.71
Brain	0.56	0.21	0.67	0.57
Spleen	0.86	0.72	0.82	0.79
Kidneys	0.31	0.65	0.76	0.75
Pancreas	0.34	0.41	0.54	0.43
Stomach (washed)	0.88	18.90	16.26	9.22
Small intestine (washed)	0.33	1.33	2.23	1.48
Muscle	0.21	0.37	0.73	0.68
Bone	0.21	0.19	0.37	0.25
Testes	0.18	0.47	0.56	0.50
Lungs	0.36	0.58	0.91	0.80

In figures 3 to 7 inclusive, the alcoholic concentration of the tissues are plotted against time and the tissue/blood concentration ratios are also plotted against time. The alcoholic concentration of the blood is plotted against time for comparison.

From the results of these experiments, it would seem that following the oral administration of 2.5 grams of ethyl alcohol per kilo body weight, the alcoholic concentration of the blood and tissues reaches a maximum in about two hours. Although the actual concentration of the blood and tissue alcohol varied considerably with the individual, there is, however, quite a definite relationship between the concentration of the alcohol in the tissues to that in the blood. This relationship is not constant in all tissues but varies with the time which elapsed following the administration of the alcohol.

In the case of the liver, lungs, spleen and kidneys, the ratio of the tissue alcoholic concentration to that of the blood reached a maximum in less than two hours and remained quite constant for at least two more hours. As shown in Fig. 3, the alcoholic concentration curves of the liver and blood are quite similar. The ratio T/B for the liver is almost constant from 0.5 to four hours after the administration of the alcohol. This value, 0.7, is in agreement with those reported in the literature (46), (68). The alcoholic concentration curves for the spleen and kidneys are similar to that for the liver and are almost identical with each other. This is shown in Fig. 4. The normal T/B ratio for the spleen, stomach, heart and brain are relatively higher than the ratios for the other

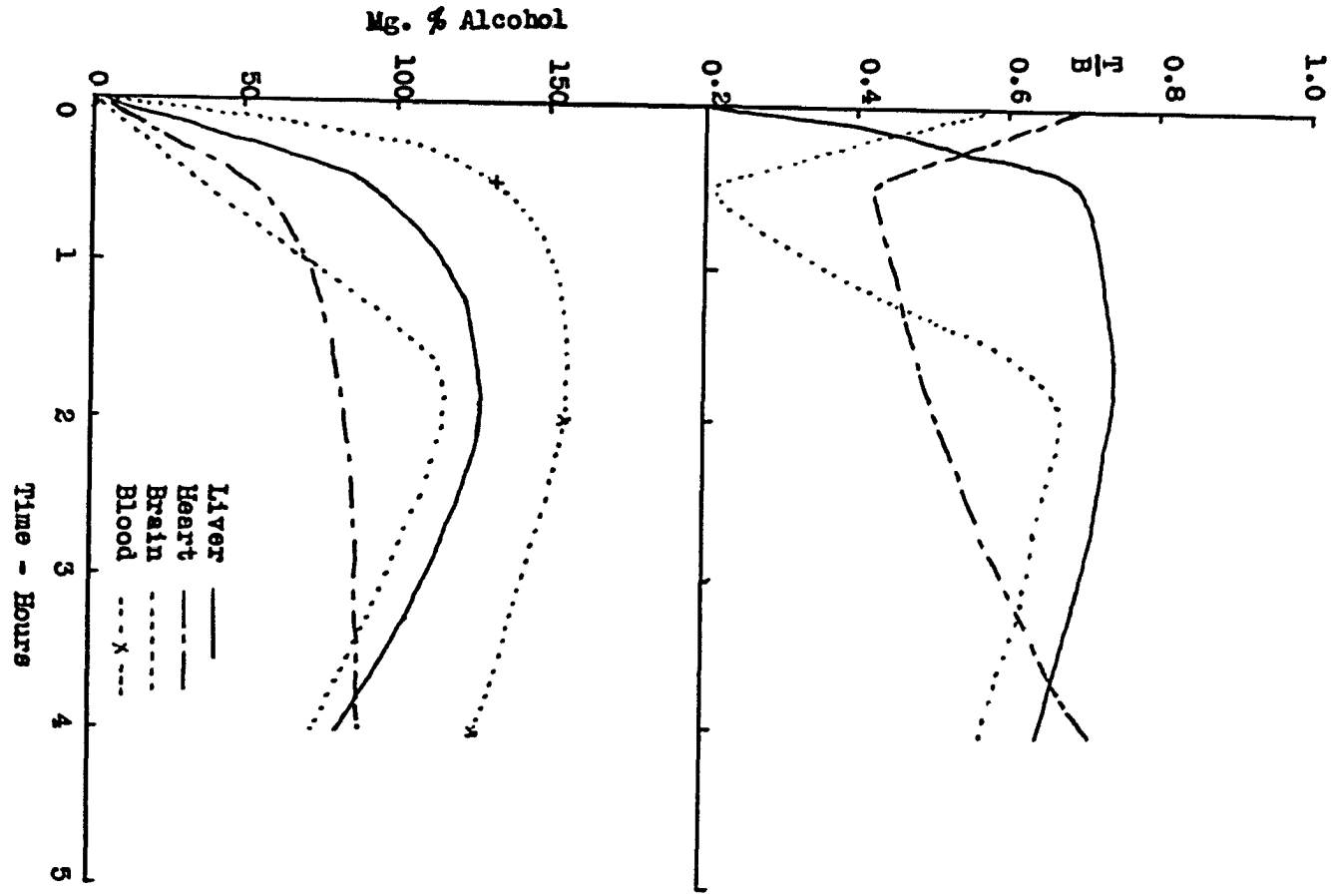

Fig. 3. The Alcoholic Concentration of the Liver, Heart and Brain Compared with that of the Blood Following the Oral Administration of Alcohol.

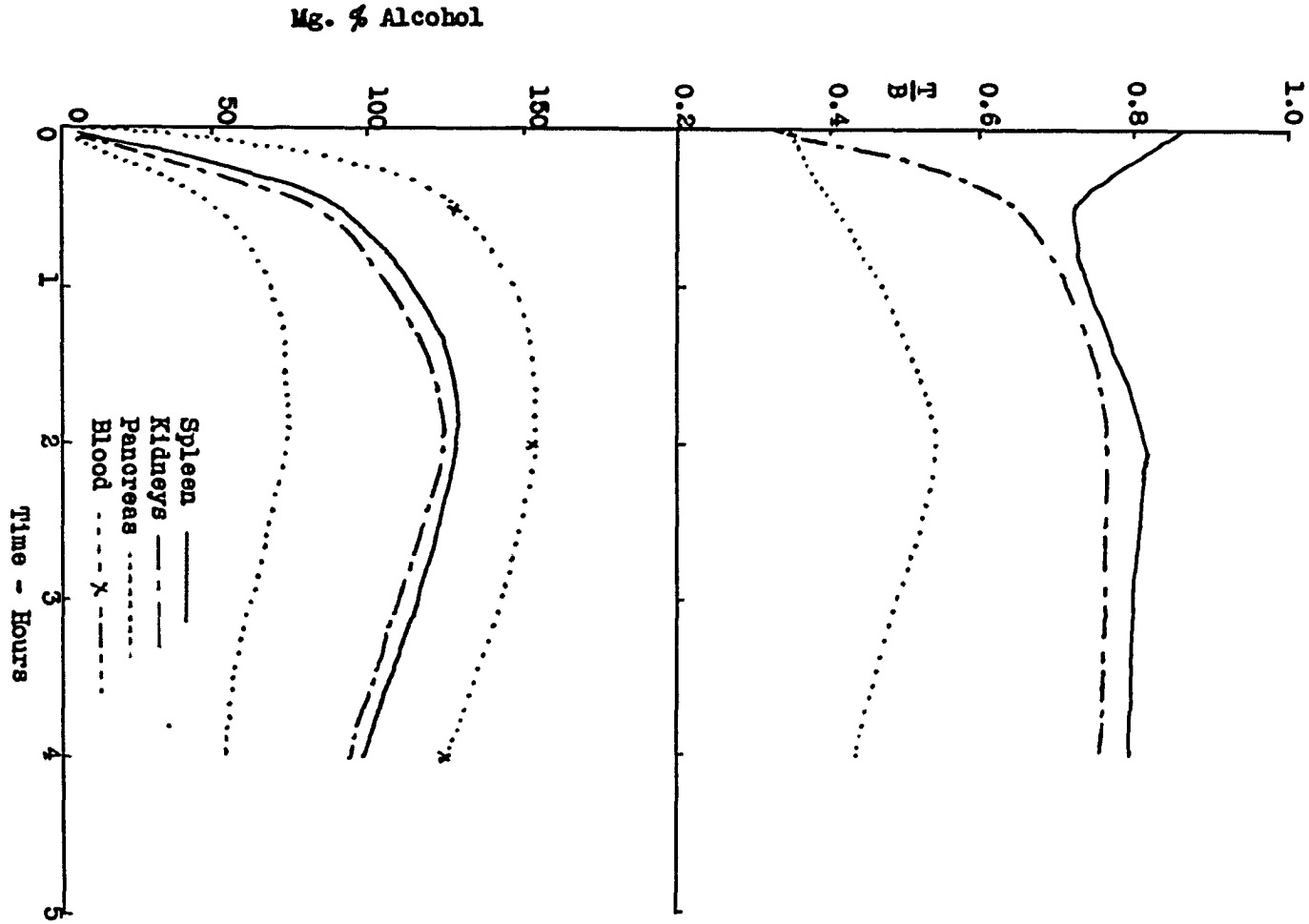

Fig. 4. The Alcoholic Concentration of the Spleen, Kidneys and Pancreas Compared with that of the Blood Following the Oral Administration of Alcohol.

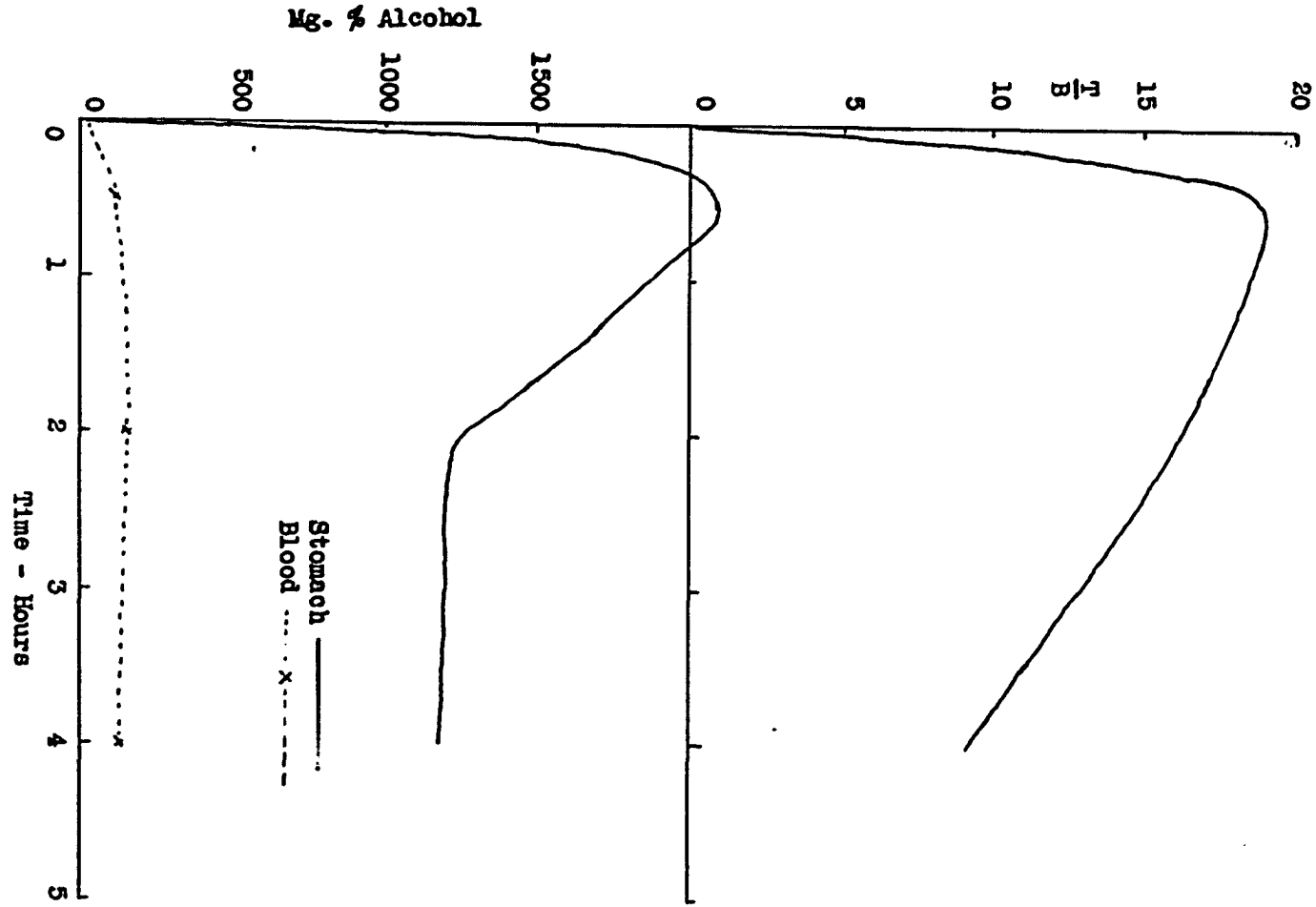

Fig. 5. The Alcoholic Concentration of the Stomach (washed) Compared with that of the Blood Following the Oral Administration of Alcohol.

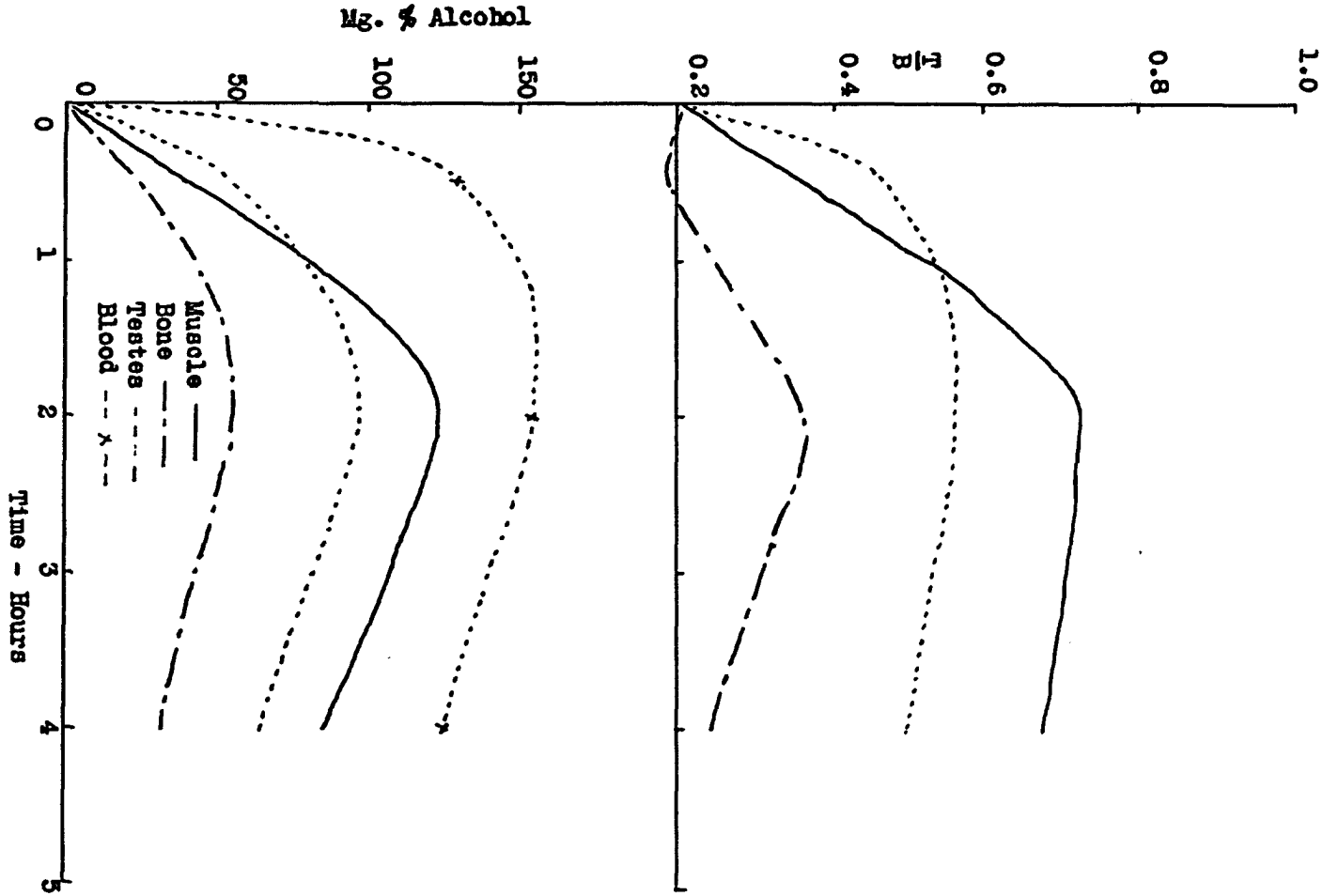

Fig. 6. The Alcoholic Concentration of the Muscle, Bone and Testes Compared with that of the Blood Following the Oral Administration of Alcohol.

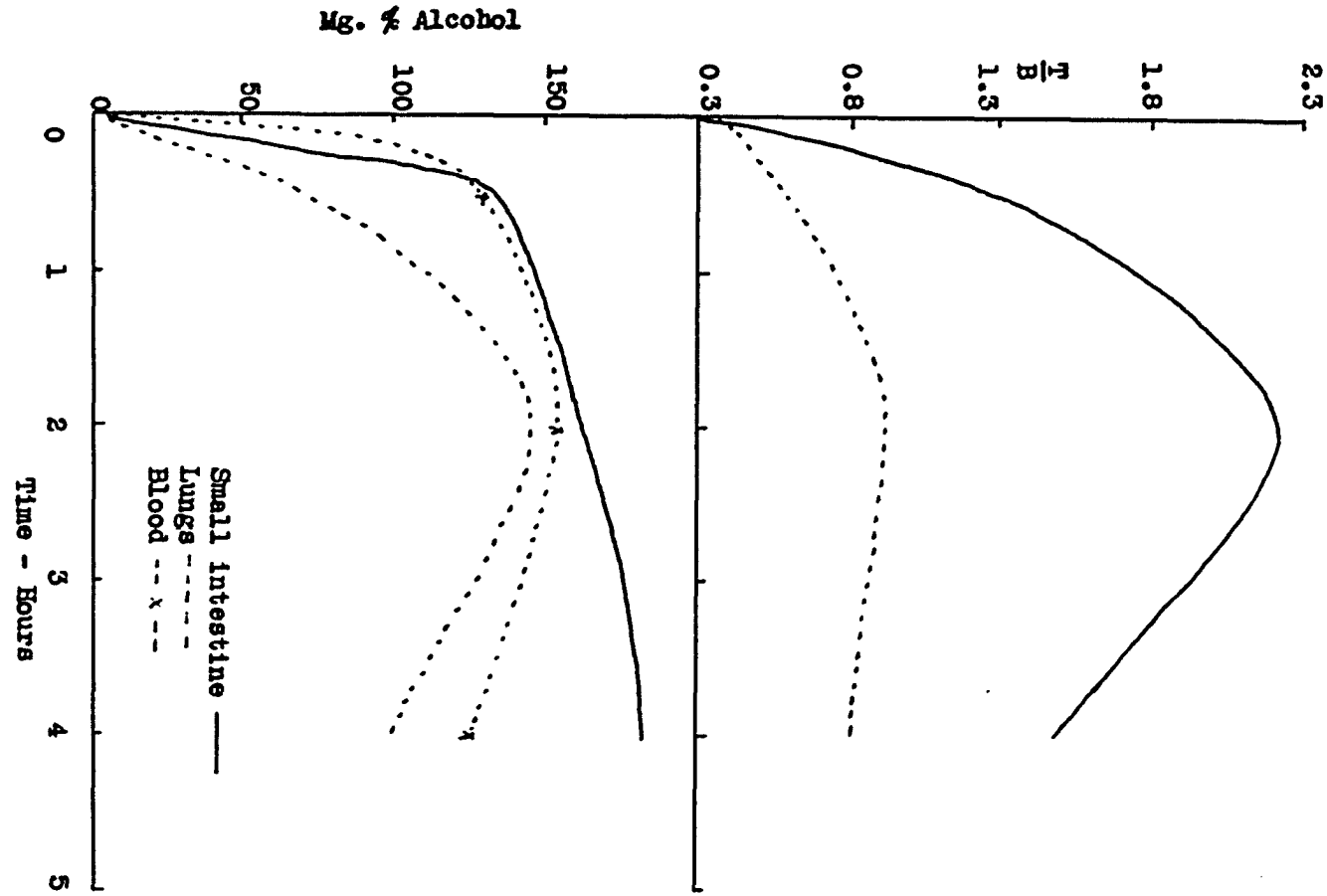

Fig. 7. The Alcoholic Concentration of the Small Intestine (washed) and the Lungs Compared with that of the Blood Following the Oral Administration of Alcohol.

tissues studied. When no alcohol was given the spleen had a normal ratio of 0.86. When alcohol was absorbed into the blood, its concentration in the spleen was such that a T/B ratio of about 0.8 was maintained for the four-hour period. The kidney had a lower T/B ratio when no alcohol was administered, but after the administration of alcohol the T/B ratio for the kidney soon reached and maintained a T/B ratio of 0.75. The lungs show about the same T/B ratio as the kidneys except the ratios for the lungs vary more with time.

The heart was the only organ studied which had not reached a maximum T/B ratio by two hours. See Fig. 3. It would be reasonable to expect the heart to behave in a manner similar to other muscle. However, the amount of blood remaining in the heart is difficult to control and although the blood was pressed from the organ, it is possible that the residual blood was responsible for its abnormal behavior. The heart did resemble the other muscle more closely than it did any other tissue.

In the study of the stomach, it was desirable to know the concentration of alcohol in the tissue and not the unabsorbed alcohol; hence the contents of the stomach were washed out before analysis for alcohol. This procedure is certain to introduce some error, since it would be impossible to prevent the loss of a certain portion of the alcohol from the tissue during the washing. The results do show that a maximum amount of alcohol is absorbed into the stomach tissue within 30 minutes and that the concentration diminishes following

that time. The concentration of alcohol in the stomach tissues appeared to remain quite constant between two and four hours following the administration of the alcohol.

The results obtained with the washed small intestine were also quite variable, since it is probable that most of the alcohol was present in the portion of the intestine immediately posterior to the stomach.

In the other organs studied, the alcohol was absorbed slower and the maximum concentration obtained was lower than the above. Contrary to the belief reported in some semi-scientific writings (29) that alcohol was concentrated in the brain, from the results obtained, it seems that alcohol is absorbed slowly and reaches a maximum T/B ratio of about 0.7 in the brain. This ratio was obtained in two hours and had diminished by four hours to about 0.6. The average ratio at 30 minutes was only 0.21 with the highest individual value of 0.35 and the lowest value of 0.14. Of the six rats studied 30 minutes after receiving 2.5 grams of ethyl alcohol per kilo body weight, three showed a T/B ratio for the brain of less than 0.2, two showed 0.24 and only one the high value of 0.35. The maximum T/B ratio obtained at two hours was 0.75, a minimum of 0.53 and an average of 0.67 was found using six rats. Three of these gave T/B ratios for the brain between 0.65 and 0.70. At four hours the ratios obtained varied more, one being 0.84 as the highest and a low of 0.30. Four of the rats, however, gave T/B ratios in the range between 0.54 and 0.60; hence the average ratio was lower at four hours

than at two hours. During that period the actual average concentration of alcohol in the brain fell from 115.0 mg. per cent to 71.4 mg. per cent. The absorption of alcohol in the brain compares best with the absorption by muscle tissue.

The absorption of alcohol by muscle is similar to the absorption by the brain, being rather slow but reaching a T/B ratio of 0.73 in two hours. The decline of the T/B ratio in the period between two and four hours is slower than that exhibited by the brain. At four hours the ratio reaches 0.68.

The pancreas and testes show a similar rate of absorption of alcohol. Both reach a maximum T/B ratio after two hours and the ratio falls slightly by four hours.

The absorption by bone is slow and the maximum T/B ratio is only 0.37 at two hours.

On the basis of these results, it is possible to establish the concentration of alcohol in the various tissues if the concentration of blood alcohol is known. If the period between 1.5 and three hours following the administration of alcohol is considered, the tissues may be grouped and approximate T/B ratios assigned for each group. These generalizations are shown in table 19.

TABLE 19

THE APPROXIMATE T/B RATIOS FOR GROUPS OF TISSUES FOR THE PERIOD OF TIME BETWEEN 1.5 AND THREE HOURS FOLLOWING THE ORAL ADMINISTRATION OF ETHYL ALCOHOL

Group	Tissue	T/B
1	Lungs	0.80
	Spleen	0.80
	Kidneys	0.75
2	Liver	0.70
	Muscle	0.70
3	Brain	0.60
	Heart	0.60
4	Testes	0.55
	Pancreas	0.50
5	Bone	0.30

No attempt can be made, on the basis of these data, to predict the alcoholic content of the stomach and small intestine tissues, since the results obtained showed too much individual variation.

The Effect of Administered Alcohol Upon Its Concentration In the Blood

The effect of orally-administered alcohol upon its concentration in the blood of both fasted and unfasted rats.

Purpose. These experiments were conducted in order to establish blood alcohol-time curves for both fasted and unfasted rats, following the oral administration of definite amounts of alcohol.

The experiments using unfasted rats were made in order to show the effect of food material in the stomach upon the absorption of alcohol into the blood. Most of these experiments were conducted for four hours or more in order to include some data following the time of maximum concentration of alcohol in the blood.

Procedure. The fasted rats were deprived of food for 24 hours preceding the administration of the alcohol. All of the rats had free access to water during the fasting period. No water was made available during the experiment. The alcohol was administered in the form of a 25 per cent solution or less by means of a stomach tube. The rats were lightly anesthetized with chloroform while the alcohol was being administered. Great care was exercised in administering the anesthetic in order to prevent damage to the liver insofar as possible.

The blood samples were all taken from the external saphenous vein; hence the data so obtained were those of venous blood.

The method used to determine the concentration of alcohol in the blood is described on page 31 of this thesis. All of the rats used in these experiments were normal animals, which had not been subjected to previous experimentation. Only mature rats were used, their ages being from 150 to 200 days and weights from 250 to 400 grams.

Results and interpretations. The results obtained in these experiments were expected to show the normal change in blood alcohol following the oral administration of various amounts of alcohol to rats.

In one series of experiments, eight fasted rats were given 2 grams of alcohol per kilo body weight. The alcohol was in a 20 per cent solution, that is, the solution contained 20 grams of alcohol per 100 ml. of solution. By use of this concentration 1 ml. of solution per 100 grams body weight gave the desired amount of alcohol. The individual blood alcoholic concentration-time curves were in very close agreement. The average results of these experiments are given in table 20.

TABLE 20

THE AVERAGE BLOOD ALCOHOL CONCENTRATION AT VARIOUS TIMES FOLLOWING THE ORAL ADMINISTRATION OF TWO GRAMS OF ALCOHOL PER KILO BODY WEIGHT TO EIGHT FASTED RATS

Time (minutes)	Blood Alcohol (mg. per cent)
0	5.0
15	60.0
30	72.0
60	88.0
90	99.0
120	103.0
150	98.0
180	83.0
210	70.0
240	53.0
270	40.0

The curve of these data is included in Fig. 8.

Good agreement was found in these experiments between individual rats indicating that the alcohol is absorbed, metabolized and eliminated at a nearly identical rate by each individual. This agreement is not so easily obtained in experiments in which higher concentrations of alcohol are given orally.

In another series of experiments, a large number of fasted rats were given 2.5 grams of ethyl alcohol per kilo body weight. The alcohol solutions administered contained 25.0 grams of alcohol per 100 ml. of solution. Thus 1 ml. was administered per 100 grams body weight. Most of the rats showed similar blood alcoholic concentration-time curves, but there were a number of exceptions which exhibited a relatively slow absorption. This slow absorption has been reported by

other investigators (39) and is due to the induction of pylorospasms by alcohol solutions of 25 per cent concentration or more. Some of the data which were obtained in these experiments were reported by Fish and Nelson (20). These data excluded those rats which showed pylorospasms as evidenced by an abnormally low blood alcoholic concentration, as much as two hours following the administration of the alcohol. The data which were reported are shown in table 21.

TABLE 21

THE EFFECT OF THE ORAL ADMINISTRATION OF 2.5 GRAMS OF ALCOHOL TO FASTED RATS UPON THE ALCOHOLIC CONCENTRATION OF THE BLOOD. THE RATS SHOWING PYLOROSPASMS WERE EXCLUDED.

Time (minutes)	Blood alcohol (mg. per cent)
0	5.0
15	94.0
30	134.0
60	170.0
90	198.0
120	206.0
150	204.0
180	198.0
210	192.0
240	182.0
270	174.0

The curve of these data is included in Fig. 8.

This curve gave a higher maximum than that found in an average curve including all of the fasted rats treated with 2.5 grams of alcohol per kilo. The data obtained from 20 rats including five which showed marked pylorospasms are shown in table 22.

TABLE 22

THE AVERAGE BLOOD ALCOHOL CONCENTRATION AT VARIOUS TIMES FOLLOWING THE ORAL ADMINISTRATION OF 2.5 GRAMS OF ETHYL ALCOHOL PER KILO TO FASTED RATS INCLUDING THOSE SHOWING PYLOROSPASM

Time (minutes)	Blood alcohol (mg. per cent)
0	5.0
15	98.7
30	129.0
60	157.0
90	179.0
120	179.0
150	171.0
180	165.0
210	158.0
240	143.0
270	135.0

The curve of these data is shown in Fig. 8.

It is obvious that pylorospasms caused the blood alcohol to remain at a lower level. Animals which showed pylorospasms had large amounts of alcohol in the stomach at the end of the experiment.

It was desirable to construct the curve from which the rats showing pylorospasms were eliminated, since such rats were not considered in other absorption studies.

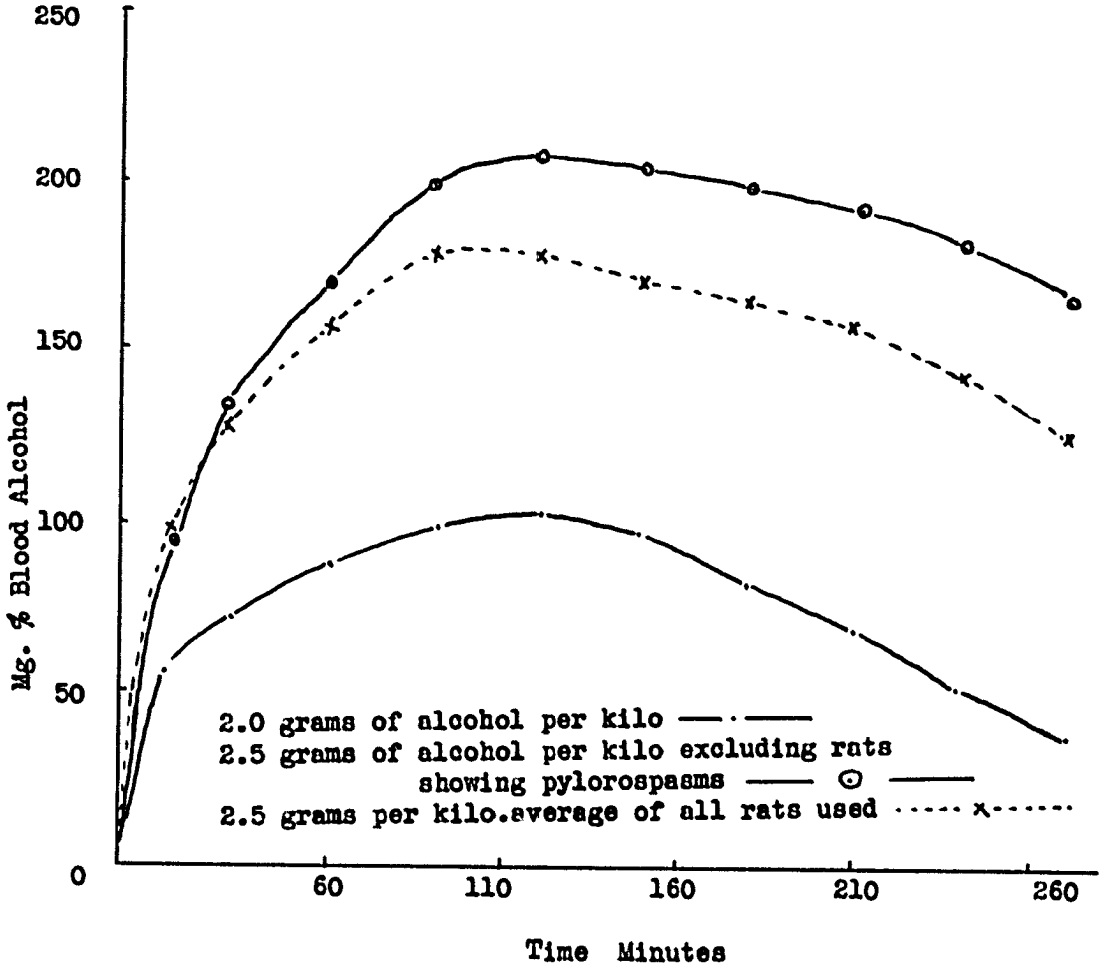

Fig. 8. The Effect of Orally Administered Alcohol Upon the Alcoholic Concentration of the Venous Blood of Fasted Rats.

The oral administration of alcohol to unfasted rats gave variable effects upon the alcoholic content of the blood. The variations were probably due to the influence of the food material present in the stomach at the time the alcohol was administered. In some instances the rats probably exhibited pylorospasms. Some of the typical results are shown in table 23.

TABLE 23

THE EFFECT OF THE ORAL ADMINISTRATION OF 2.5 GRAMS OF ALCOHOL PER KILO BODY WEIGHT UPON THE ALCOHOLIC CONTENT OF THE BLOOD OF UNFASTED RATS

Time (minutes)	Mg. per cent alcohol in venous blood			
	Rat 1	Rat 2	Rat 3	Rat 4
0	5	5	5	5
15	90	58	98	88
30	185	118	120	52
60	218	125	108	98
90	224	134	90	152
120	208	147	78	180
150	190	170	52	163
180	170	200	-	140
210	150	220	-	116
240	-	-	-	92

The curves of these data are shown in Fig. 9.

From these experiments it would appear that alcohol solutions up to 20.0 per cent or slightly more, do not cause pylorospasms; hence good agreement may be obtained between individual rats given the same amount of alcohol per kilo body weight if the rats have been

previously fasted. The maximum concentration of alcohol in venous blood was attained at two hours, following the oral administration of 2.0 or 2.5 grams of alcohol per kilo to fasted rats.

The unfasted rats attained a maximum concentration of alcohol in the venous blood at various times, depending upon the influence of the food material present in the stomach when the alcohol was given. Rat 3, Fig. 9, probably had a pronounced pylorospasm which caused an early maximum in the curve. Rat 2 may have had a temporary pylorospasm, the effect of which was to delay the maximum. Food material present in the stomach caused a further delay. Rat 4 reached a maximum blood alcoholic concentration in two hours, but the rise in concentration was slow at first, which was due to the influence of food material in the stomach. Rat 1 gave a curve quite similar to that of a fasted rat given the same treatment. The stomach of this rat contained very little food material at the end of the experiment.

No average curve could be given for the unfasted rats, since the absorption was influenced not only by pylorospasms, but by the nature and amount of food present in the stomach at the start of the experiment.

Although no experiments were conducted in an attempt to determine the rate of metabolism, some of the results shown seem to indicate a constant rate of decrease in the alcoholic concentration of venous blood. This cannot be interpreted as indicating a constant

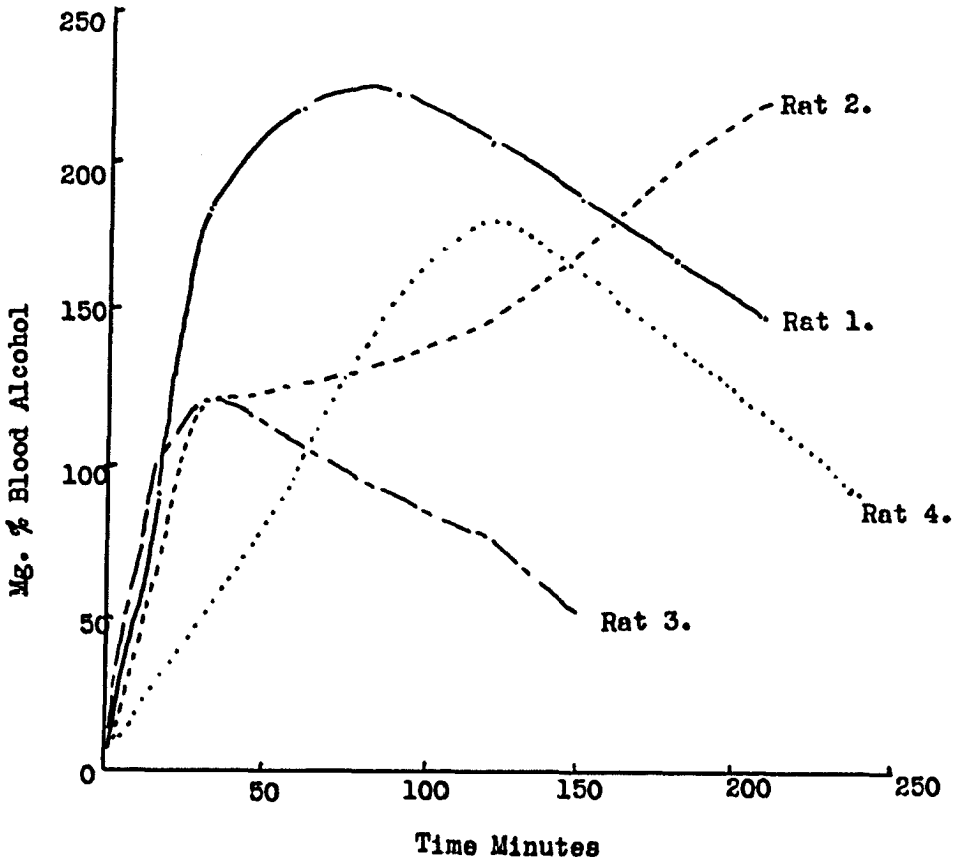

Fig. 9. The Effect of the Oral Administration of 2.5 Grams of Alcohol per Kilo Body Weight, to Unfasted Rats, Upon the Concentration of Alcohol in Venous Blood.

rate of metabolism, however. To do so, the following assumptions would have to be made: (a) the rate of elimination is constant and (b) absorption and distribution are completed by the time the rate of decrease of blood alcohol becomes constant. The decline in the blood alcoholic concentration is due to the difference between amount of alcohol lost through metabolism and elimination and gained by absorption. The oral administration of 2.5 grams of alcohol per kilo to fasted rats gave a curve which follows the equation of a straight line 150 minutes after its administration. The equation for that portion of the curve is as follows:

$$y = -0.483x + 171.4$$

where:

y = concentration of alcohol in mg. per cent

x = time in minutes

The factor -0.483 is the rate of decrease in the concentration of the venous blood alcohol in mg. per cent per minute. It must be emphasized that this equation is valid only after 150 minutes and only if 2.0 grams of alcohol per kilo body weight are administered to fasted rats. The rats used were nearly all the same weight and age, which may have been factors influencing the rates of absorption, elimination and metabolism of the alcohol.

It is not possible to derive a straight line equation for any portion of the curves obtained when 2.5 grams of alcohol per kilo are administered orally to fasted rats. The administration of 2.5 grams

of alcohol per kilo probably induces pylorospasms of varying degrees, hence interfering with absorption.

The influence of absorption upon the blood alcohol-time curve is clearly demonstrated in the experiments, using unfasted rats. The effects of the administration of 2.5 grams of alcohol per kilo upon the alcoholic concentration of the venous blood of four rats are shown in Fig. 9. It is possible to derive straight line equations for a portion of the curves obtained from rats 1, 3 and 4. These equations are as follow:

Rat 1, $y = -0.66x + 288$

Rat 3, $y = -0.61x + 143$

Rat 4, $y = -0.80x + 283$

where:

y = concentration of alcohol in mg. per cent

x = time in minutes

In the case of Rat 2, Fig. 9, a case of delayed absorption is clearly demonstrated. Some food material was found in the stomach after the experiment was terminated. In addition to the effect of the food, there may have been a temporary pylorospasm. The rat was obviously intoxicated during the last 30 minutes of the experiment, but no signs of intoxication could be observed early in the experiment.

The effect of habituation upon the absorption of orally administered alcohol into the blood.

Purpose. These experiments were conducted in order to determine the influence of continued use of alcohol upon the blood alcohol-time curve obtained following the oral administration of definite amounts of alcohol to fasted rats.

There is some disagreement in the literature as to the reason for the fact that a habituated individual can ingest relatively more alcohol, without becoming intoxicated, than a non-habituated individual.

Some investigators reported that the alcohol was oxidized more rapidly in the tissues of a habituated indvdual. Other nvestigators reported that the tissues of a habituated individual are less sensitive to alcohol; hence it would require more alcohol to produce intoxication. If the latter reason is correct, the blood alcohol-time curves should be approximately the same for habituated and non-habituated indviduals given a similar treatment. These experiments were conducted to determine if the blood alcohol-time curves were changed by habituation.

Procedure. One group of rats was habituated by being given a 10 per cent alcohol solution to drink instead of water from the weaning age of 28 days until the rats were 120 days of age. These rats were then fasted 24 hours and given water to drink during the fasting period. Two levels of alcohol were administered orally to the fasted

rats; some were given 2.0 grams per kilo of body weight and the others 2.5 grams per kilo of body weight.

Another group of eight normal rats was selected which were 165 days of age. These rats were given 2.5 grams of alcohol per kilo of body weight after fasting 24 hours. The blood alcohol-time curves were obtained and served as the normal for comparison. The rats were then given a 10 per cent alcohol solution instead of water to drink for 120 days. Following this habituation period, the rats were fasted 24 hours and then given 2.5 grams of alcohol per kilo of body weight.

Results and interpretations. The average results obtained by giving 2.0 grams of alcohol per kilo of body weight to six fasted rats from the group which were habituated during the growing period of from 28 to 120 days of age are shown in table 24.

TABLE 24

THE EFFECT OF THE ORAL ADMINISTRATION OF 2.0 GRAMS OF ALCOHOL PER KILO OF BODY WEIGHT TO FASTED RATS, WHICH HAD BEEN HABITUATED TO ALCOHOL, UPON THE ALCOHOLIC CONCENTRATION OF VENOUS BLOOD

Time (minutes)	Blood alcohol (mg. per cent)
0	5.0
15	62.0
30	73.0
60	90.0
90	100.0
120	105.0
150	101.0
180	85.0
210	72.0
240	55.0
270	41.0

The curves of these data and the data which were obtained in similar experiments on non-habituated rats are shown in Fig. 10.

Eight rats of the same group of habituated rats were given 2.5 grams of alcohol per kilo after fasting 24 hours. One of the rats developed a pronounced pylorospasm and therefore was excluded from the results which are shown in table 25.

TABLE 25

THE EFFECT OF THE ORAL ADMINISTRATION OF 2.5 GRAMS OF ALCOHOL PER KILO TO FASTED RATS, WHICH HAD BEEN HABITUATED TO ALCOHOL, UPON THE ALCOHOLIC CONCENTRATION OF VENOUS BLOOD.

Time (minutes)	Blood alcohol (mg. per cent)
0	5.0
15	90.0
30	130.0
60	168.0
90	195.0
120	203.0
150	201.0
180	196.0
210	190.0
240	181.0
270	175.0

The curves of these data and the data which were obtained in similar experiments on non-habituated rats are shown in Fig. 11.

The group of eight rats which were habituated after reaching the age of 165 days were given 2.5 grams of alcohol per kilo, after fasting 24 hours, both before and after a habituation period of

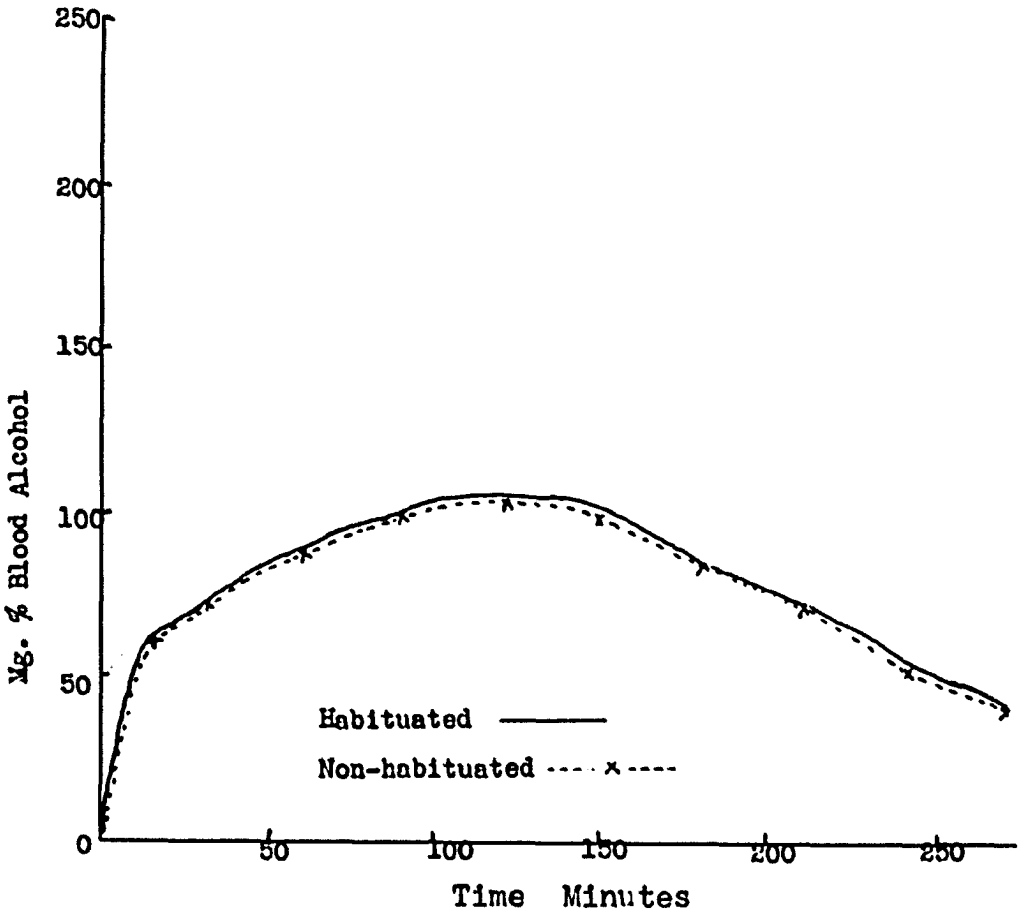

Fig. 10. The Effect of the Oral Administration of 2.0 Grams of Alcohol per Kilo Body Weight to Fasted Rats, Both Habituated and Non-habituated to Alcohol, Upon the Alcoholic Concentration of Venous Blood,

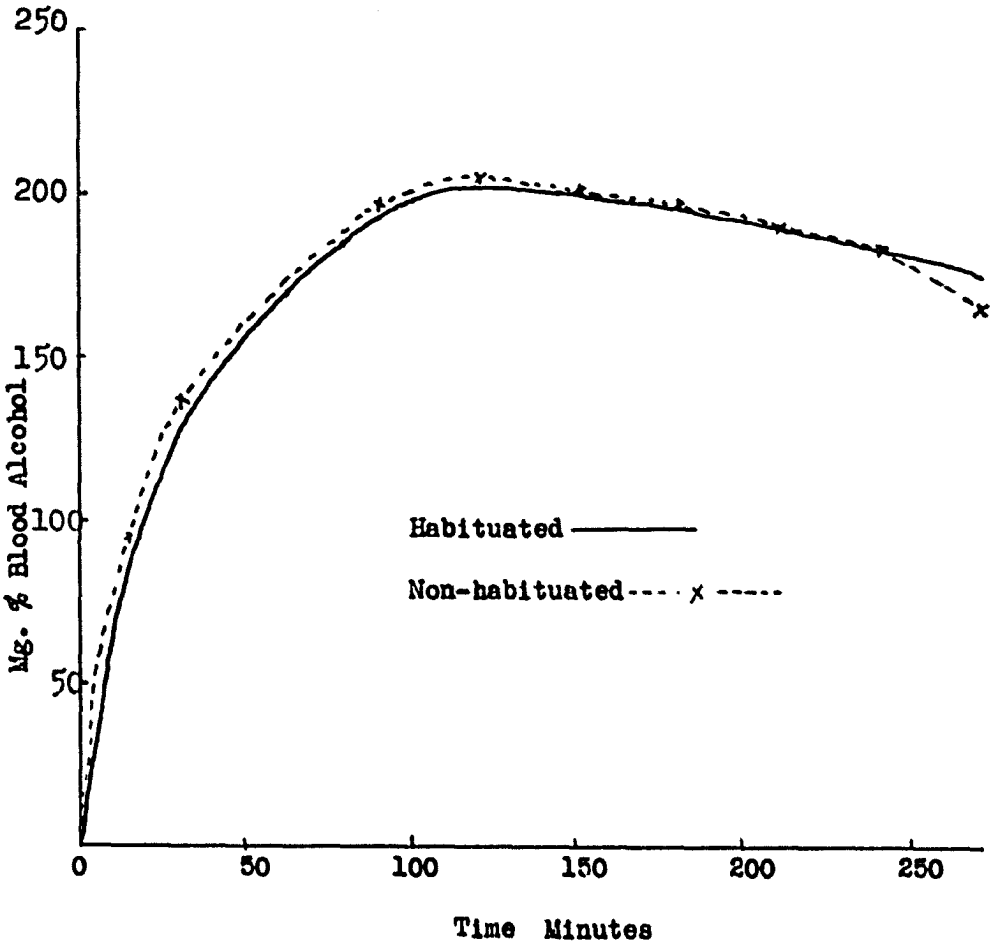

Fig. 11. The Effect of the Oral Administration of 2.5 Grams of Alcohol per Kilo Body Weight to Fasted Rats, Both Habituated and Non-habituated, Upon the Alcoholic Concentration of Venous Blood.

120 days. The average data are shown in table 26. One rat developed a pylorospasm in the experiment before habituation and is excluded from the normal average.

TABLE 26

THE EFFECT OF THE ORAL ADMINISTRATION OF 2.5 GRAMS OF ALCOHOL PER KILO TO FASTED RATS, BOTH BEFORE AND AFTER HABITUATION, UPON THE ALCOHOLIC CONCENTRATION OF VENOUS BLOOD.

Time (minutes)	Blood alcohol (mg. per cent)	
	Before habituation	After habituation
0	5.0	5.0
15	96.0	98.0
30	130.0	132.0
60	168.0	170.0
90	192.0	192.0
120	202.0	204.0
150	200.0	199.0
180	195.0	193.0
210	185.0	186.0
240	178.0	179.0
270	163.0	165.0

The curves of these data are shown in Fig. 13.

From the results found in these experiments, it seems that habituation had no influence upon the effect of orally administered alcohol to fasted rats on the concentration of alcohol appearing in venous blood. These results lend support to the belief that habituation to alcohol develops a tissue tolerance, rather than a change in the rate of oxidation, absorption or elimination of the alcohol.

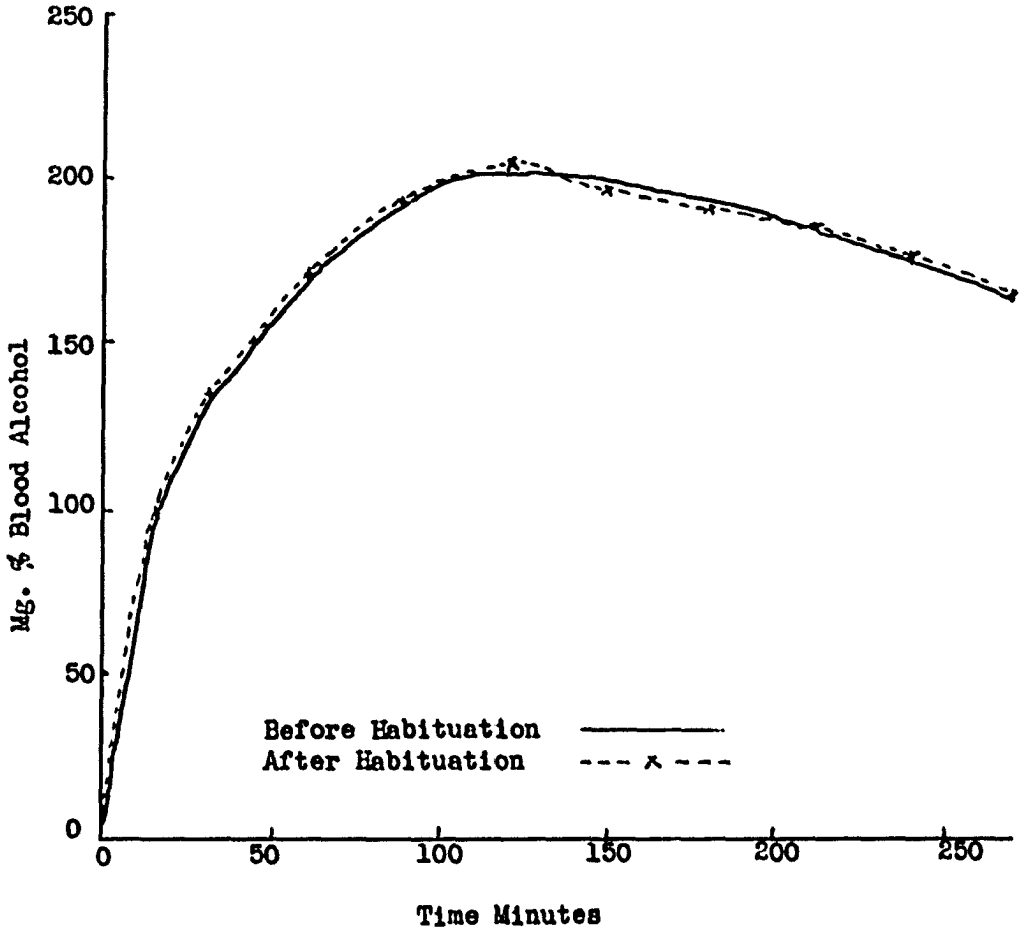

Fig. 12. The Effect of the Oral Administration of 2.5 Grams of Alcohol per Kilo Body Weight to Fasted Rats, Both Before and After Habituation, upon the Alcoholic Concentration of Venous Blood.

The effect of the intraperitoneal injection of alcohol upon the concentration of alcohol in the venous blood of rats.

Purpose. These experiments were performed in order to show the change in concentration of alcohol in venous blood following the intraperitoneal injection of various amounts of alcohol.

Procedure. Definite amounts of alcohol were injected into the abdominal cavity about 1 inch posterior to the liver and slightly to the right of the median line. The concentration of alcohol in the venous blood was determined at various times until the concentration had dropped considerably. Normal rats which had not previously received alcohol were used in these experiments. Alcohol solutions were injected so that 1.25, 1.41, 1.86 and 2.50 grams of alcohol per kilo body weight were administered.

Results and interpretations. The average data obtained at each level used in these experiments are shown in table 27. The curves of these data are shown in Fig. 13.

Good agreement was obtained between the individual rats receiving similar amounts of alcohol. This is probably due to the rapid establishment of equilibrium between the blood and the contents of the peritoneal cavity. The absorption is not complicated by differences in the time of evacuation of the stomach as it is with orally administered alcohol.

From these results it would appear that alcohol is very rapidly absorbed from the peritoneal cavity, since a maximum concentration is reached in the blood within 15 to 20 minutes.

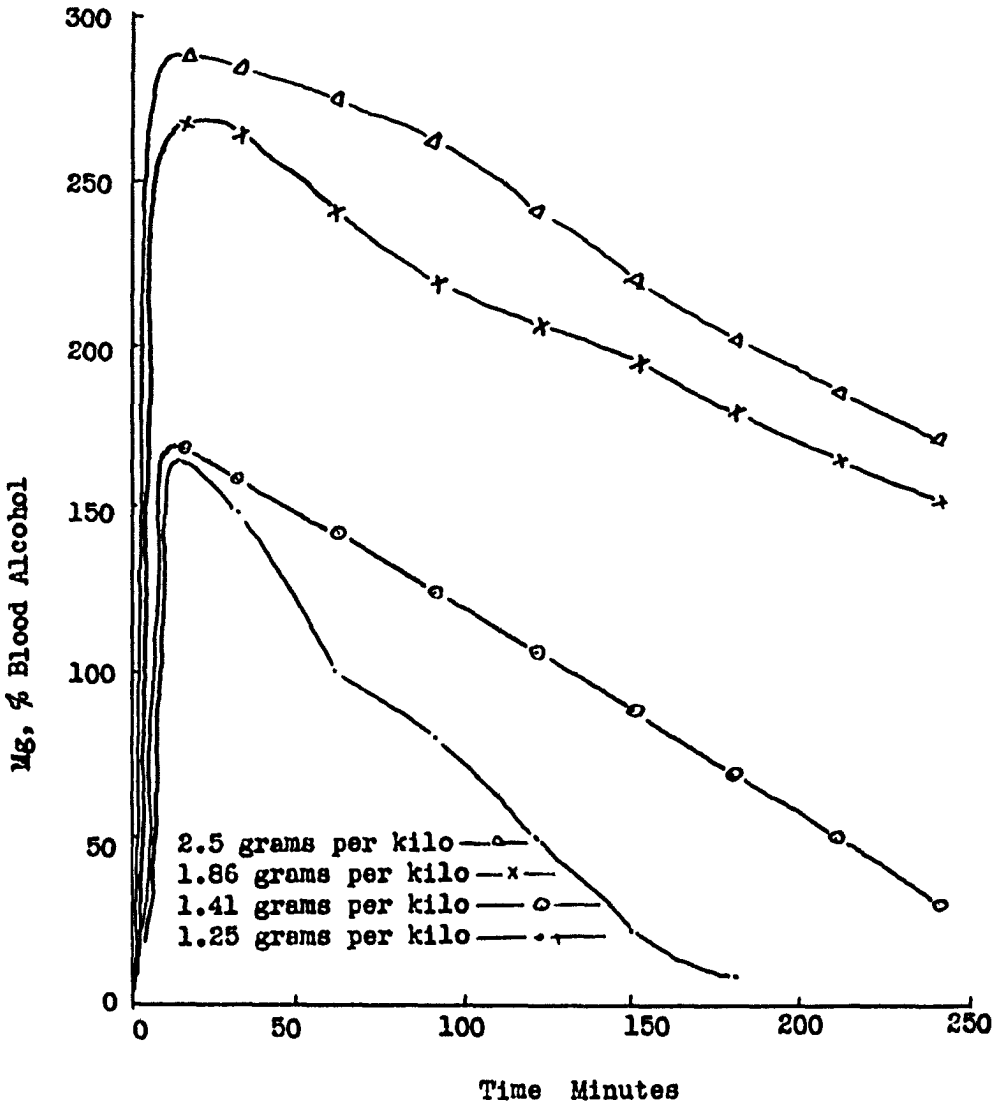

Fig. 13. The Effect of the Intraperitoneal Injection of Alcohol upon the Concentration of Alcohol in the Venous Blood.

TABLE 27

THE EFFECT OF THE INTRAPERITONEAL INJECTION OF ALCOHOL UPON THE ALCOHOLIC CONCENTRATION IN VENOUS BLOOD

Time (minutes)	Mg. per cent alcohol Alcohol administered in grams per kilo			
	1.25	1.41	1.86	2.50
0	5.0	5.0	5.0	5.0
15	132.0	168.0	268.0	288.0
30	148.0	158.0	266.0	236.0
60	100.0	142.0	240.0	276.0
90	80.0	124.0	220.0	264.0
120	50.0	107.0	208.0	240.0
150	20.0	88.0	196.0	220.0
180	8.5	76.0	180.0	202.0
210	-	52.0	165.0	186.0
240	-	30.0	152.0	172.0

The curves shown in Fig. 13 indicate that the declining portion of each curve is nearly a straight line. With the exception of the curve obtained from rats receiving 1.25 grams of alcohol per kilo, the slopes of the lines are nearly the same.

The equation for the declining portion of the average curve for the rats receiving 1.41 grams per kilo is as follows:

$$y = -0.61x + 180$$

where:

y = concentration of blood alcohol in mg. per cent

x = time in minutes

The rate of decrease of the blood alcohol in this case was 0.61 mg. per cent per minute. Nearly the same rate of decrease was found for the rats receiving 1.86 and 2.50 grams per kilo. This rate of

decrease of blood alcohol is probably more nearly related to the rate of metabolism than the rates of decrease in blood alcohol found after the oral administration of alcohol.

This closer relationship is possible since absorption from the peritoneal cavity is much more rapid than from the digestive tract. This fact would permit a faster distribution in the tissues than is the case in oral administration.

It is difficult to study the effect of the injection of 2.5 grams of alcohol per kilo of body weight or more, since the higher concentrations of alcohol cause a rapid development of a comatose state and a lowered blood pressure which makes it difficult to obtain blood samples.

The effect of the oral administration of some substances along with the alcohol upon its absorption into the blood.

Purpose. It has been reported in the literature that milk inhibits the absorption of alcohol from the digestive tract. These experiments were conducted in order to investigate the influence of milk, skim milk, cream and glucose upon the absorption of alcohol from the digestive tract.

Procedure. Normal rats were fasted 24 hours and were then given 2.5 grams of alcohol per kilo of body weight which was dissolved in a solution of the substance under investigation. The venous blood was analyzed for alcohol at the usual intervals of time. The average blood-time curves so obtained were compared with the normal curve.

Results and interpretations. A group of eight normal rats was selected and, after fasting 24 hours, each rat was given 2.5 grams of alcohol and 5.2 ml. of whole milk per kilo body weight. The alcoholic concentration of the venous blood was determined at various times. The average results are shown in table 28.

TABLE 28

THE EFFECT OF WHOLE MILK UPON THE ABSORPTION OF ORALLY ADMINISTERED ALCOHOL INTO THE BLOOD

Time (minutes)	Mg. per cent alcohol
0	5.0
15	80.0
30	107.0
60	116.0
90	123.0
120	123.0
150	124.0
180	118.0
210	114.0
240	102.0
270	89.0

The curve of these data is included in Fig. 14.

Two groups of normal rats, six in each group, were selected and, after being fasted 24 hours, were treated as follows: Group 1 received 2.5 grams of alcohol and 5.2 ml. of skim milk per kilo body weight, and group 2 received 2.5 grams of alcohol and 5.2 ml. of cream, 50 per cent butter fat, per kilo body weight. The alcoholic concentration of the venous blood was determined as usual. The results of these

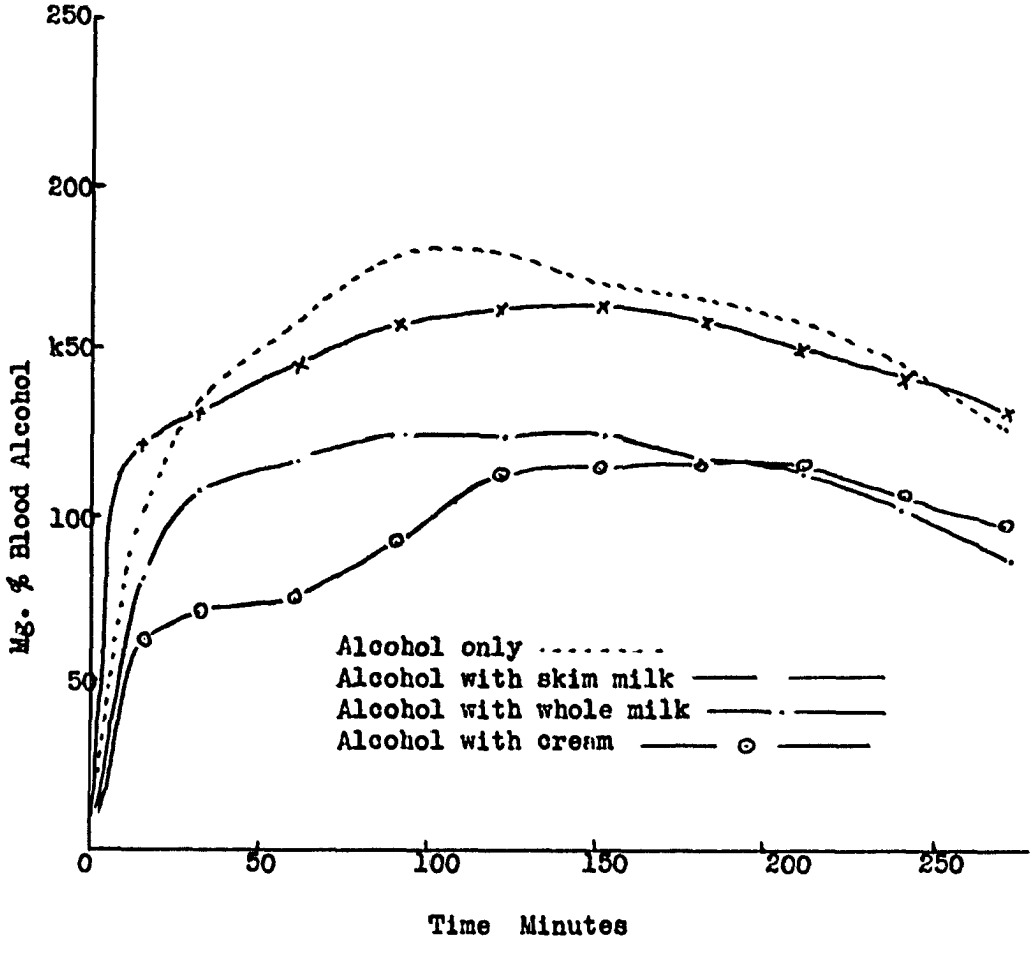

Fig. 14. The Influence of Whole Milk, Skim Milk and Cream Upon the Absorption of Alcohol from the Digestive Tract into the Blood.

experiments are shown in table 29.

TABLE 29

THE EFFECT OF SKIM MILK AND CREAM UPON THE ABSORPTION OF ORALLY ADMINISTERED ALCOHOL INTO THE BLOOD

Time (minutes)	Mg. per cent alcohol	
	Skim milk	Cream
0	5.0	5.0
15	119.0	61.0
30	129.0	70.0
60	143.0	75.0
90	156.0	92.0
120	161.0	112.0
150	162.0	115.0
180	158.0	117.0
210	150.0	115.0
240	141.0	107.0
270	131.0	99.0

The curves of these data are included in Fig. 14.

In order to determine the influence of glucose upon the absorption of alcohol from the digestive tract, a group of four normal rats was treated as follows: Each rat was fasted 24 hours and then given 2.5 grams of alcohol and 1 gram of glucose per kilo body weight.

The alcoholic concentration of the venous blood was determined at various intervals of time. The results are shown in table 30.

TABLE 30

THE INFLUENCE OF GLUCOSE UPON THE ABSORPTION OF ORALLY ADMINISTERED ALCOHOL INTO THE BLOOD

Time (minutes)	Mg. per cent alcohol
0	5.0
15	90.0
30	136.0
60	167.0
90	176.0
120	181.0
150	182.0
180	181.0
210	166.0
240	148.0
270	128.0

The curve of these data is shown in Fig. 15.

From these results it seems that glucose does not inhibit the absorption of alcohol from the digestive tract. Skim milk has a slight inhibitory action upon the absorption of alcohol. Whole milk has a definite inhibitory action upon absorption and cream containing 50 per cent butter fat has a very pronounced inhibitory action during the first two hours. After that time, however, the cream and whole milk have about the same influence upon the absorption of alcohol. The inhibitory action of whole milk depends largely upon its fat content, since the fat-free milk has almost no influence. The slight inhibitory action of skim milk is probably due to its protein content.

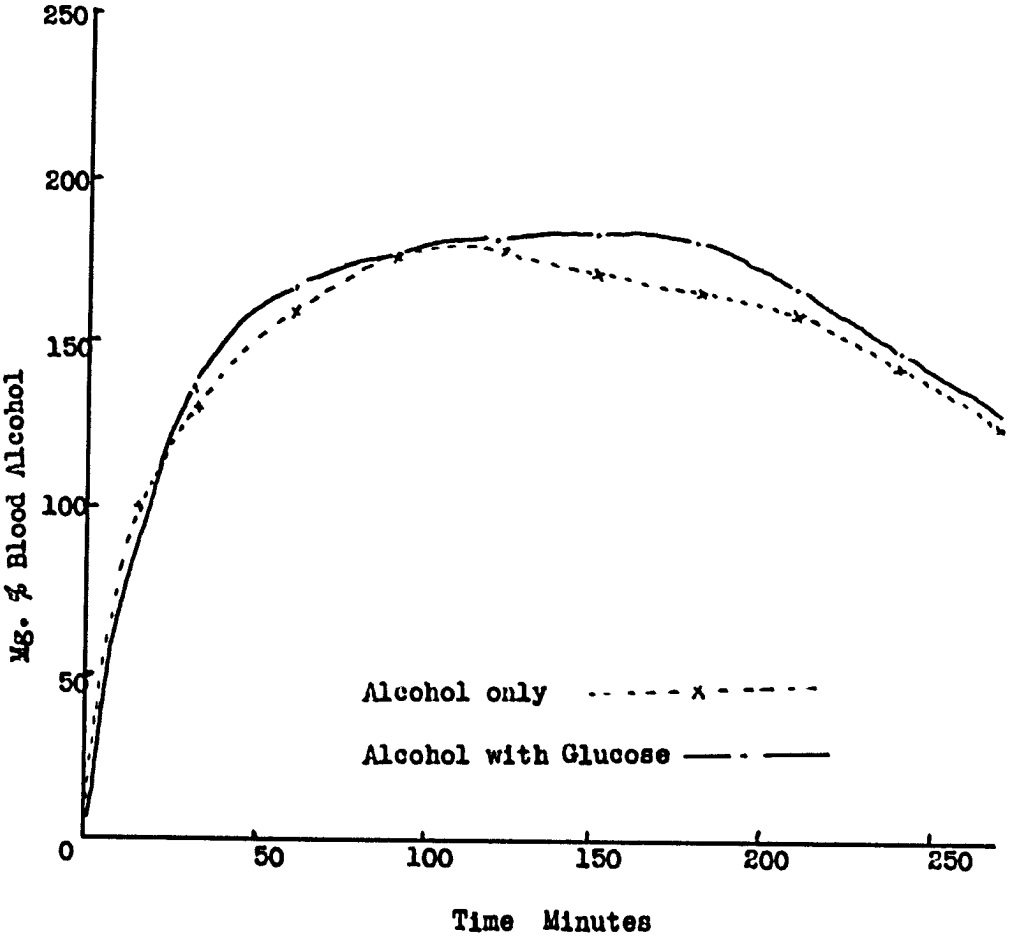

Fig. 15. The Influence of Glucose Upon the Absorption of Alcohol from the Digestive Tract into the Blood.

The Effect of Alcohol Upon Growth and Feed Consumption

Purpose.

These experiments were conducted in order to obtain some information regarding the influence of alcohol upon the growth of rats. It was desirable to have some information as to the amount of alcohol which could be ingested without seriously influencing normal growth.

Procedure.

Young normal rats were selected weighing about 50 grams. These rats were divided into groups consisting of an equal number of males and females in each group. Ten were selected for each group. In one experiment four groups were used consisting of five males and five females in each. Each group was given all of the stock ration they would eat. A record was kept of the weight of each individual rat and the amount of feed and drink consumed by each group. Group 1 was given distilled water to drink; group 2 was given 1 per cent alcohol instead of water; group 3 was given 5 per cent alcohol instead of water and group 4 was given 10 per cent alcohol instead of water to drink.

Another group of 10 rats, group 5, was selected consisting of five males and five females, the males and females being kept separated in order to avoid the influence of pregnancy upon the weights of the females. These rats averaged about 50 grams in weight

at the start of the experiment. They were given stock ration to eat and alcohol solutions to drink as follows: one per cent alcohol for the first month, 5 per cent the second and 10 per cent alcohol the third month and thereafter. Records were kept of weight, food consumption and alcohol consumption. In addition to growth studies these rats were to be used to study the effect of alcohol upon certain normal blood constituents.

Results and interpretations.

The growth curves obtained in these experiments are shown in figures 16, 17 and 18.

A record of food and liquid consumption was made over a period of 91 days. These data are shown in table 31.

From these results it would seem that alcohol solutions up to 10 per cent could be substituted for drinking water for most of the rats without causing any marked inhibition of growth. The female rats grew at a subnormal rate and some died. The 10 per cent alcohol solution caused the greatest inhibition of growth during the first month of the experimental period. When the concentration of alcohol was increased gradually as it was in group 5, the rats gained as well as those receiving no alcohol. The use of 10 per cent alcohol solutions instead of water resulted in a poor nutritional state among the female rats if used over a period of from five to six months.

TABLE 31

FOOD AND LIQUID CONSUMPTION AND GROWTH OF RATS RECEIVING VARIOUS CONCENTRATIONS OF ALCOHOL INSTEAD OF WATER TO DRINK OVER A 91-DAY PERIOD

Group	% alcohol solutions consumed	Ml. liquid consumed per rat	Grams of alcohol ingested per rat	Grams of food consumed per rat	Average gain in weight per rat		No. of deaths
					Males	Females	
1	0.0	2042	0.0	936.5	179	132	1
2	1.0	1600	16.0	948.5	183	120	0
3	5.0	1382	69.1	765.7	165	109	0
4	10.0	1122	112.2	737.0	163	115*	2*
5	1.0 to 10.0	1668	93.7	1169.0	203	138	0

* The two rats which died in group 4 were females. Two of the three which lived had shown a growth rate much above the average of the group of five females, while all were alive.

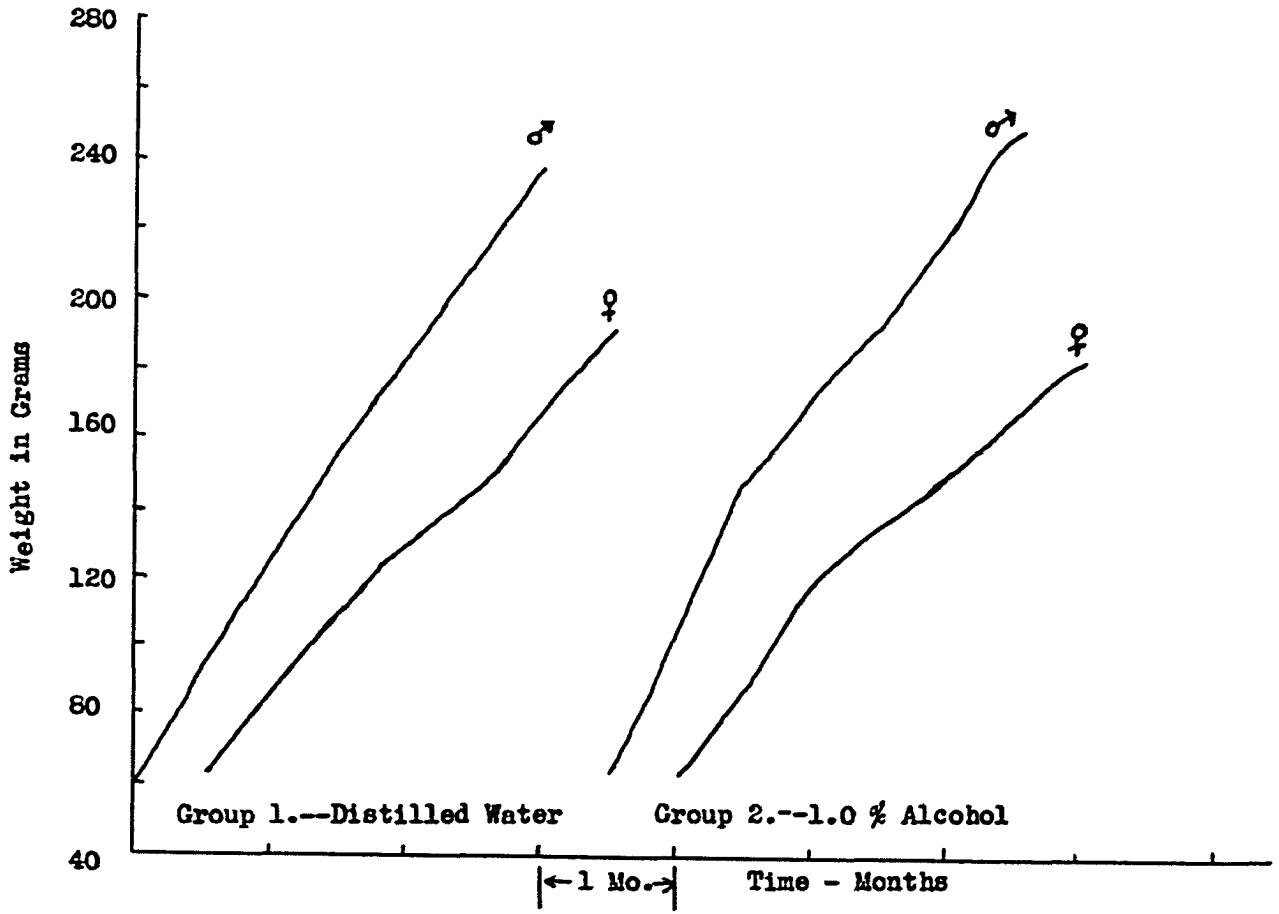

Fig. 16. The Average Growth of Rats Receiving 0.0 and 1.0 Per Cent Alcohol to Drink.

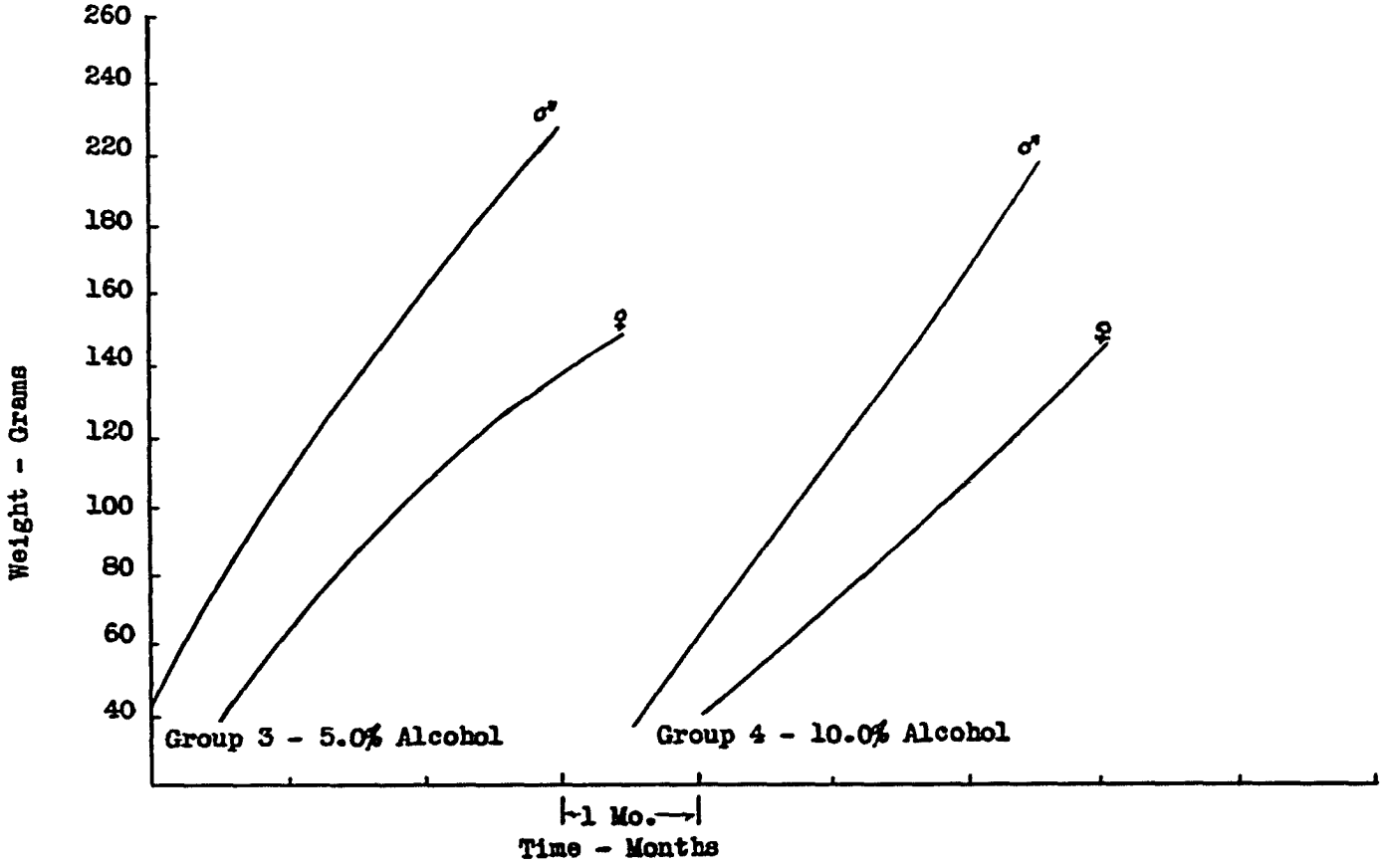

Fig. 17. The Average Growth of Rats Receiving 5.0 and 10.0 Per Cent Alcohol to Drink Instead of Water.

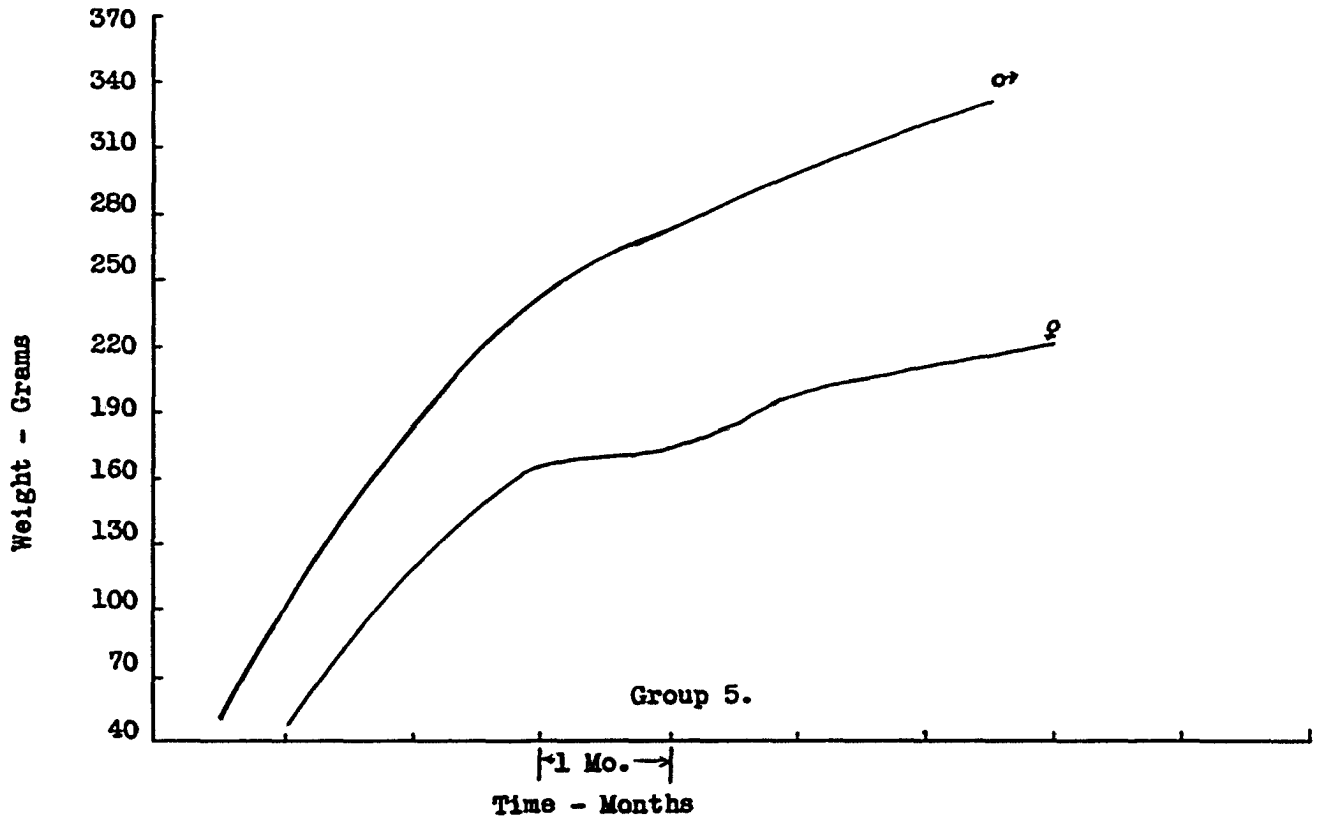

Fig. 18. The Average Growth of Rats Receiving Alcohol Solutions to Drink as Follows: First Month, 1.0 Per Cent, Second Month, 5.0 Per Cent, and 10.0 Per Cent Thereafter.

Since the rats receiving alcohol instead of water grew nearly as well as those receiving water, and at the same time showed a lower feed intake, it would seem that alcohol will take the place of food in the diet to a limited extent.

The use of 1.0 per cent alcohol did not decrease food intake but at the same time the rats showed a faster growth rate than normal. The rats receiving 5.0 per cent alcohol showed a diminished food intake but showed a greater gain per unit of food consumed, which is at least partially due to the food value of the alcohol.

The Effect of Ingested Alcohol Upon Certain Normal Blood Constituents

Purpose.

Since the use of alcohol has been reported to be directly or indirectly responsible for certain pathological changes in the liver, digestive tract and possibly the kidneys, it seemed desirable to find whether or not such changes would affect certain normal blood constituents. Those blood constituents to be studied were selected which could be determined quantitatively using a small blood sample. In this way it was possible to study the same rat at various times, thereby following any changes which might occur.

Procedure.

Two groups of normal adult rats, 5.5 months of age, were selected consisting of four males in one group and four females in the other. The following blood constituents were determined before any alcohol was given: hemoglobin, uric acid, non-protein nitrogen and the blood sugar level following a fasting period of 36 hours. These same blood constituents were determined 30, 60 and 90 days following the substitution of 10 per cent alcohol for water to drink. The rats were given water to drink only during the 36-hour fasting period.

Two other groups of rats were selected at the age of one month. One group consisted of five males and the other of five females. These rats were given alcohol solutions instead of water to drink. During the first month these rats received 1.0 per cent alcohol, 5.0 per cent the second month and 10 per cent from then on until they reached the age of 5.5 months. At that age the same blood constituents were determined as were previously stated.

Results and interpretations.

In reporting the results, the males and females in the groups receiving 10 per cent alcohol after 5.5 months of age will be designated as group A, while the groups receiving increasingly concentrated alcohol solutions following the age of one month will be designated as group B. The concentrations of hemoglobin, uric acid, non-

protein nitrogen and glucose in the blood of group B rats were determined when these rats reached the age of 5.5 months, in order that they could be compared with the concentrations of the same substances as determined for group A before alcohol was given them.

The results of the experiments on the concentration of hemoglobin are given in table 32.

TABLE 32

THE AVERAGE EFFECT OF ALCOHOL UPON THE CONCENTRATION OF HEMOGLOBIN IN RATS' BLOOD

Group	Number of days alcohol was given				
	0	30	60	90	165
A males	14.9	15.8	15.1	14.8	-
A females	14.5	13.4	14.7	14.6	-
B males	-	-	-	-	16.7
B females	-	-	-	-	16.0
Average of both sexes	14.7	14.6	14.9	14.7	16.3

The results of the experiments on the effect of alcohol upon the concentration of uric acid in the blood of rats are shown in table 33.

TABLE 33

THE AVERAGE EFFECT OF ALCOHOL UPON THE CONCENTRATION OF URIC ACID IN RATS' BLOOD

Group	Number of days alcohol was given				
	0	30	60	90	165
A males	1.68	2.01	1.84	1.88	-
A females	1.43	1.32	1.46	1.70	-
B males	-	-	-	-	1.43
B females	-	-	-	-	1.37
Average of both sexes	1.55	1.67	1.65	1.79	1.40

The average results of the experiments on the effect of alcohol upon the non-protein nitrogen of the blood are given in table 34.

TABLE 34

THE AVERAGE EFFECT OF ALCOHOL UPON THE CONCENTRATION OF NON-PROTEIN NITROGEN IN RATS' BLOOD

Group	Number of days alcohol was given				
	0	30	60	90	165
A males	41.8	46.8	40.5	46.2	-
A females	44.8	46.0	40.1	42.1	-
B males	-	-	-	-	48.0
B females	-	-	-	-	44.5
Average of both sexes	43.3	46.4	40.3	44.2	46.2

The results obtained in the experiments on the effect of alcohol upon the fasting blood sugar level following a fasting period of 36 hours are given in table 35.

TABLE 35

THE AVERAGE EFFECT OF ALCOHOL UPON THE CONCENTRATION OF GLUCOSE IN THE BLOOD OF RATS FOLLOWING A 36-HOUR FASTING PERIOD

Group	Number of days alcohol was given				
	0	30	60	90	165
A males	105.0	95.1	98.5	94.3	-
A females	96.4	87.9	82.5	89.8	-
B males	-	-	-	-	85.9
B females	-	-	-	-	89.7
Average of both sexes	100.7	91.5	90.2	92.0	87.8

The only apparent change in the concentration of the normal blood constituents caused by the ingestion of alcohol in these experiments was a slight decrease in the blood sugar fasting level. Since there were no changes in the concentration of uric acid or non-protein nitrogen, it seems certain that no extensive damage was done to the kidney by alcohol under these conditions.

Any change in the hemoglobin content of the blood might have been a result of nutritional anemia. No such changes were caused by alcohol in the amounts given.

DISCUSSION

The methods used in this investigation were developed largely from those reported in the literature. They were, in general, developed in such a way that they could be used in connection with studies involving rats as experimental animals. In the analysis of the blood of rats, it was necessary to use a small sample of 0.1 ml. or less in most instances. By the use of such samples the same individual could be used in several related experiments or a number of successive samples could be taken from the same individual. It was thus possible to obtain very satisfactory blood alcohol curves without removing an excessive amount of blood from the rat.

The only blood vessels which are easily accessible in the rat are the external saphenous veins or the peripheral vessels of the tail. The blood from the external saphenous vein was used most often in these experiments, hence the data apply to venous blood. When arterial blood was desirable in single samples, it was removed from the heart by means of a hypodermic needle. No more than two samples could be taken from the same individual in this way. A more satisfactory investigation of the absorption, distribution and metabolism of alcohol would have been possible if a satisfactory source of arterial blood had been available.

All of the methods used in this investigation were satisfactory and simple. However, the method used for the determination of uric acid in blood could not be made to check the standard methods. Good

agreement was obtained between check samples of the same blood; hence the method was satisfactory for this work in which any changes in the concentration of uric acid in the blood were to be detected. This method cannot be recommended for use in the determination of the absolute concentration of uric acid in the blood of rats. In the development of the method, it seemed that there was some relationship between the values obtained and the pH of the protein-free filtrate. A great deal of care was used to insure that the pH of all samples were the same. All of the methods of blood analysis used in this investigation could be used as reported for work on man unless the absolute uric acid value was desired.

This investigation was not planned to determine the rate of metabolism of alcohol; however, it did disclose a few facts which should be recognized in any investigation of metabolism of alcohol. If alcohol is administered orally, solutions should be used which are dilute enough so as not to cause pylorospasms. It seemed that pylorospasms had a variable influence upon the absorption of alcohol and that would seriously interfere with studies on metabolism. The intraperitoneal injection of alcohol would seem to be more satisfactory since absorption is much more rapid and is probably more consistent.

On the basis of this work it seemed that the distribution of alcohol in the tissues is not complete until about two hours following

the oral administration of alcohol. If alcohol is administered intraperitoneally the distribution in the tissues should be more rapidly completed. The results obtained regarding the distribution of alcohol in the tissues are summarized in table 19.

The elimination of alcohol from the body was not studied in this investigation, since there seemed to be good agreement in the literature as to the amount lost from the body in this way. In fact, there is believed to be such a direct relationship between the alcoholic content of expired air and the blood that it is possible to determine the blood alcohol indirectly from the concentration of alcohol in expired air.

No direct reports dealing with the effect of ingested alcohol upon the normal blood constituents other than alcohol were found in the literature. From the results of the experiments dealing with this subject, it was found that alcohol in the amounts given caused no definite changes in such constituents as were studied. The only possible exception was the blood sugar level following a fasting period of 36 hours. This change may be explained as being a result of a loss of glycogen from the liver which results in a diminished carbohydrate store in the body. Even this effect is not very pronounced under the conditions used.

The study regarding the normal blood constituents was limited to those constituents which could be determined in a very small sample of blood and therefore is far from complete. Some work was

done on a method by which blood urea could be determined in 0.1 ml. of blood. No such method was found, however, that was satisfactory. It was not desirable to devote a great deal of time to the development of such a method, since any marked changes in blood urea would have been evidenced by changes in the non-protein nitrogen concentration.

Some of the methods used in this work may be valuable to other fields of study. The method by which very dilute standard solutions of ethyl alcohol were prepared might be applied, with certain variations, to the preparation of dilute solutions of other volatile substances.

It is also possible that the titration method developed by Harger (42) and used in this work for the determination of potassium dichromate might be applied to the determination of chromium and vanadium in steel and other samples. The end point in the titration is very sharp and the reagents used are easily prepared and standardized.

CONCLUSIONS

As a result of the present investigations of the determination of ethyl alcohol in the blood and tissues, its absorption and distribution and its effect upon some of the blood constituents of the rat, the following conclusions have been derived:

1. A satisfactory method by which the alcoholic concentration of blood may be determined using 0.1 or 0.05 ml. of sample has been developed and its accuracy proven. The method is also simple and rapid and may be used on either freely-flowing or oxalated blood.
2. A method has been developed by which the alcoholic concentration of the tissues may be determined. The method was designed to prevent the loss of alcohol during manipulation. The distribution of alcohol in 12 different tissues has been studied and the relationship between the concentration of alcohol in the tissues and the concentration in the blood has been established. These distribution ratios were determined under conditions in which no alcohol had been administered and 30 minutes, two hours and four hours following the oral administration of 2.5 grams of ethyl alcohol per kilo body weight.
3. Blood alcohol concentration curves have been established using venous blood following the oral administration of definite amounts of alcohol to both fasted and unfasted

rats. It was found that 2.5 grams of alcohol per kilo body weight caused pylorospasms in several instances. The oral administration of 2.0 grams of alcohol per kilo body weight did not induce pylorospasms. If pylorospasms did not result, quite consistent results were obtained using fasted rats. The results using unfasted rats were inconsistent due to the influence of food material in the stomach.

4. Habituation to alcohol had no influence upon the blood alcohol-time curves obtained following the oral administration of alcohol solutions.

5. The absorption of alcohol from the peritoneal cavity is very rapid and high blood alcohol levels are reached within 15 minutes. The rate of decrease in blood alcohol is quite consistent at levels of injected alcohol of 1.41, 1,86 and 2.50 grams per kilo body weight.

6. Whole milk and cream inhibit the absorption of alcohol from the digestive tract. Glucose at a level of 1.0 gram per kilo body weight has no inhibitory action on the absorp- of alcohol from the digestive tract and skim milk has only a slight effect.

7. The use of 5.0 and 10 per cent alcohol solutions instead of water decreased the amount of food consumed by rats. Male rats

showed better growth than female rats while receiving alcohol solutions to drink instead of water. All of the rats which died while receiving 10 per cent alcohol were females.

8. The substitution of 10 per cent alcohol for drinking water had no effect upon the hemoglobin, uric acid and non-protein nitrogen concentration of the blood. The fasting blood sugar level was slightly decreased by the use of 10 per cent alcohol.

SUMMARY

A micro method was developed and used in these experiments by which the alcoholic concentration of blood could be determined. The method proved to be rapid, simple and accurate.

The addition of 0.15 per cent of a mixture of equal parts by weight of sodium fluoride and potassium oxalate will prevent coagulation and act as a preservative for blood samples to be used in alcohol determinations.

A method has been developed by which the alcoholic concentration of tissues may be determined. The chances for loss of alcohol during manipulation have been minimized by means of an apparatus developed for this investigation.

The distribution of alcohol in the tissues has been investigated. The relationship between the concentration of alcohol in the tissues to that in the blood has been established. It was not constant but changed with time following the oral administration of alcohol. The ratios found for the period between 1.5 and three hours are quite constant. The muscles, brain and fatty tissue attain equilibrium with the blood rather more slowly than other tissues.

The effect of orally administered alcohol upon its concentration in the venous blood of rats has been studied using fasted and unfasted rats. With fasted rats, the maximum concentration of alcohol in venous blood was reached in about two hours.

The oral administration of 2.5 grams of alcohol per kilo of body weight, using a 25 per cent solution, caused pylorospasms in several rats. The administration of 2.0 grams per kilo body weight, using a 20 per cent solution, did not cause pylorospasms. The average curve for all rats studied, using 2.0 grams of alcohol per kilo body weight, showed a constant rate of decrease in the blood alcohol after 150 minutes.

The oral administration of alcohol solutions to unfasted rats gave variable results. Food material in the stomach inhibited the absorption of alcohol.

Habituation of rats to alcohol had no effect upon the blood alcohol curves obtained following the oral administration of alcohol solutions.

Alcohol was very rapidly absorbed into the blood from the peritoneal cavity. The resulting blood alcohol curves showed about the same rate of decrease. This would be a good means of administration of alcohol in metabolism studies since absorption is more uniform. Since the absorption of alcohol was so rapid from the peritoneal cavity, a maximum concentration was reached in 15 minutes following its adminstration. The intraperitoneal injection of 2.5 grams of alcohol caused the development of coma and a rapid fall in blood pressure. For this reason it was difficult to obtain satisfactory blood samples from the external saphenous vein. Injection levels of 1.86 or less grams of alcohol per kilo of body weight were

easily studied.

Of the substances studied whole milk and cream had the greatest inhibitory action upon the absorption of alcohol from the digestive tract. Skim milk had a slight inhibitory action while glucose in the amount of 1.0 gram per kilo body weight had no effect. Most of the inhibitory influence of milk was due to its fat content while the protein fraction probably had a slight influence.

The substitution of 1.0, 5.0 and 10 per cent alcohol solutions for drinking water did not decrease the growth rate of male rats to any marked degree. The use of 10 per cent alcohol solution did cause the death or retard the growth of female rats. A rather poor nutritional state was quite noticeable among the female rats after receiving 10 per cent alcohol for a period of six months.

The use of 5.0 or 10 per cent alcohol resulted in a diminished food consumption by the rats.

The continued ingestion of 10 per cent alcohol solutions for as long as 165 days had no effect upon the concentration of hemoglobin, uric acid and non-protein nitrogen in the blood of rats. The fasting blood sugar level was slightly diminished.

LITERATURE CITED

1. Abels, J. C. A simple method for determination of ethyl alcohol in blood. Proc. Soc. Expt'l. Biol. Med. 34:346. 1936.

2. Bavis, D. F. Tests for concentration of alcohol in blood. J. Am. Med. Assoc. 115:73. 1940.

3. Beazell, J. M. and Ivy, A. C. The influence of alcohol on the digestive tract. Quart. J. Studies Alc. 1:45. 1940.

4. Beeman, J. Blood alcohol determination. A modification of the Cettler method. Am. J. Clin. Path., Tech. Suppl. 4:45. 1940.

5. Bernhard, C. G. and Goldberg, L. Aufnahme und Verbrennung des Alkohols bei Alkoholikern. Acta Med. Scand. 86:152. 1935. Original not seen. Abstracted in Chem. Abst. 30:769. 1936.

6. Bogen, E. Tolerance to alcohol: its mechanism and significance. Calif. West. Med. 44:262. 1936.

7. Burger, M. The effects of alcohol on the normal and pathologic kidney. Quart. J. Studies Alc. 1:85. 1940.

8. Carpenter, T. M. The metabolism of alcohol: a review. Quart. J. Studies Alc. 1:201. 1940.

9. Carpenter, T. M. Human metabolism with onemata of alcohol, dextrose and levulose. Publ. Carneg. Instn. No. 369. 1925.

10. Carpenter, T. M. Ethyl alcohol in fowls after exposure to alcohol vapor. J. Pharmacol. 37:217. 1929.

11. Carpenter, T. M. and Babcock, E. B. The concentration of alcohol in the tissues of hens after inhalation. Am. J. Physiol. 49:128. 1919.

12. Cavett, J. W. The determination of alcohol in blood and other body fluids. J. Lab. Clin. Med. 23:543. 1938.

13. Clark, B. B., Morrissey, R. W., Fazekas, J. F. and Welch, C. S. The role of insulin and the liver in alcohol metabolism. Quart. J. Studies Alc. 1:663. 1941.

14. Cori, C. F., Villiaume, E. L. and Cori, G. T. Studies on intestinal absorption. II The absorption of ethyl alcohol. J. Biol. Chem. 87:19. 1930.

15. Decker, H. Über die interferometrische Bestimmung des Blutalkoholgehaltes. Deut. Z. ges. gerichtl. Med. 33:33. 1940. Original not seen. Abstracted in Chem. Zentr. 111, I:3303. 1940.

16. Dybing, O. and Rasmussen, E. Die Resorption des Äthylalkohol aur dem Mogen und Darm der Ratte. Biochem. Z. 306:337. 1940. Original not seen. Abstracted in Chem. Abstr. 35:2195. 1941.

17. Eggleston, G. Determination of the metabolic rate of alcohol. J. Physiol. 98:228. 1940.

18. Fish, V. B. and Nelson, V. E. A micro method for the determination of ethyl alcohol in blood. Proc. Iowa Acad. Sci. 48:207. 1941.

19. Fish, V. B. and Nelson, V. E. The distribution of alcohol in the tissues of the rat. Proc. Iowa Acad. Sci. In press.

20. Fish, V. B. and Nelson, V. E. The effect of oral and intraperitoneal adminstration of alcohol upon the concentration of alcohol in the blood of the rat. Proc. Iowa Acad. Sci. In press.

21. Fleming, R. and Stotz, E. Experimental studies in alcoholism. Arch. Neurol. Psychiat. 33:492. 1935.

22. Folin, O. Standardized methods for the determination of uric acid in unlaked blood and in urine. J. Biol. Chem. 101:111. 1933.

23. Folin, O. and Malmros, H. An improved form of Folin's micro method for blood sugar determinations. J. Biol. Chem. 83:115. 1929.

24. Friedemann, T. E. and Klaas, R. The determination of ethyl alcohol. J. Biol. Chem. 115:551. 1938.

25. Gettler, A. O. and Freireich, A. W. The nature of alcohol tolerance. Am. J. Surg. 27:328. 1935.

26. Gettler, A. O. and Freireich, A. W. Determination of alcohol intoxication during life by spinal fluid analysis. J. Biol. Chem. 92:199. 1931.

27. Gettler, A. O., Niederl, J. B. and Benedetti-Pichler, A. A. The isolation of pure anhydrous ethyl alcohol from non-alcoholic human and animal tissue. J. Am. Chem. Soc. 54:1476. 1932.

28. Gibson, J. G., 2nd. and Blotner, H. The determination of ethyl alcohol in blood and urine with the photoelectric colorimeter. J. Biol. Chem. 126:551. 1938.

29. Gray, G. W. The advancing front of medicine. New York, London, Whittlesey House, McGraw-Hill Book Company, Inc. 1941.

30. Goubau, R. M. Micro-dosage d'alcool dans de sang par spektrophotométrie. Bull. Acad. Med. Belg. Ser. 6, 4:196, 301. 1939. Original not seen. Abstracted in Chem. Zentr. 111, I:3433. 1940.

31. Greenberg, L. A. and Keator, F. W. A portable automatic apparatus for the indirect determination of alcohol in the blood. Quart J. Studies Alc. 2:57. 1941.

32. Gréhant, N. Construction de courbes qui indiquent les proportions d'alcool que renferme le sang apres l'injection dans l'estomac de volumes déterminés d'alcool éthylique: applications. Compt. rend. Soc. biol. 51:946. 1899.

33. Gréhant, N. and Quinquaud, E. Sur l'absorption des vapeurs d'alcool absolu par les poumons. Compt. rend. Soc. biol. 35:426. 1883.

34. Haggard, H. W. and Greenberg, L. A. Studies in the absorption, distribution and elimination of ethyl alcohol. I. The quantitative determination of ethyl alcohol in air, blood and urine by means of iodine pentoxide. J. Pharmacol. 52:137. 1934.

35. Haggard, H. W. and Greenberg, L. A. Studies in the absorption, distribution and elimination of ethyl alcohol. III. Rate of oxidation of alcohol in the body. J. Pharmacol. 52:167. 1934.

36. Haggard, H. W. and Greenberg, L. A. Absorption, distribution and elimination of alcohol. V. Influence of glycine on the absorption of alcohol. J. Pharmacol. 68:482. 1940.

37. Haggard, H. W., Greenberg, L. A. and Cohen, L. H. Quantitative differences in the effect of alcoholic beverages. New Engl. J. Med. 219:466. 1938.

38. Haggard, H. W., Greenberg, L. A. and Lolli, G. The absorption of alcohol with reference to its influence on the concentration of alcohol appearing in the blood. Quart. J. Studies Alc. 1:684. 1941.

39. Haggard, H. W., Greenberg, L. A. and Rakieten, N. Absorption distribution and elimination of alcohol. VI. Principles governing the concentration of alcohol in the blood and the concentration causing respiratory failure. J. Pharmacol. 69:252. 1940.

40. Hansen, K. Zur Theorie der Narkose. Untersuchen über die Verteilung der indifferenten Narkotika in dem tierschen Organismus. Oslo, Olaf Norli. 1925.

41. Hanzlik, P. J. and Collins, H. J. Quantitative study on the gastro-intestinal absorption of drugs. III. The absorption of alcohol. J. Pharmacol. 5:185. 1913.

42. Harger, R. N. A simple micro method for the determination of alcohol in biologic material. J. Lab. Clin. Med. 20:746. 1935.

43. Harger, R. N. Chemical tests of intoxication. J. Am. Med. Assoc. 114:1687. 1940.

44. Harger, R. N., Bridwell, E. C. and Raney, B. B. An aerometric method for the rapid determination of alcohol in water and body fluids. J. Biol. Chem. 128:38p. 1939.

45. Harger, R. N. and Goss, A. L. The so-called normal alcohol of the body. Am. J. Physiol. 112:374. 1935.

46. Harger, R. N., Hulpieu, H. R. and Lamb, E. B. The speed with which various parts of the body reach equilibrium in the storage of ethyl alcohol. J. Biol. Chem. 120:689. 1937.

47. Harger, R. N., Lamb, E. B. and Hulpieu, H. R. A rapid chemical test for intoxication employing breath. A new reagent for alcohol and a procedure for estimating the concentration of alcohol in the body from the ratio of alcohol to carbon dioxide in the breath. J. Am. Med. Assoc. 110:779. 1938.

48. Heise, H. A. The specificity of the test for alcohol in body fluids. Am. J. Clin. Path. 4:182. 1934.

49. Heise, H. A. Chemical tests for alcoholic intoxication. J. Am. Med. Assoc. 114:1391. 1940.

50. Hinsberg, K. Blutalkohol - Bestimmung. Chem. Ztg. 62: 145. 1938.

51. Hinsberg, K. and Bruetel, E. Ein kritischer vergleich zwischen den Methoden nach Widmark und nach Friedemann-Klaas zur Bestimmung des Athylalkohols im blut. Deut. Z. ges. gerichtl. Med. 31:194. 1939. Original not seen. Abstracted in Chem. Zentr. 111, I:1084. 1940.

52. Jetter, W. W. Studies in alcohol. II Experimental feeding of alcohol to non-alcoholic individuals. Am. J. Med. Sci. 196:487. 1938.

53. Jetter, W. W. Diagnosis of alcoholic intoxication. J. Am. Med. Assoc. 114:2402. 1940.

54. Jetter, W. W. A critical survey of various chemical methods for determining the alcohol content of body fluids and tissues with their physiological and medicolegal significance. Quart. J. Studies Alc. 2:512. 1941.

55. Jetter, W. W. and Forrester, C. C. The perchlorate method for determining the concentration of alcohol in expired air as a medicolegal test. Arch. Path. 32:828. 1941.

56. Jetter, W. W., Moore, M. and Forrester, C. C. A new method for the determination of breath alcohol. Am. J. Clin. Path., Tech. Suppl. 11:75. 1941.

57. Jellinek, E. M. and Jolliffe, N. Effects of alcohol on the individual: review of the literature of 1939. Quart. J. Studies Alc. 1:110. 1940.

58. Johnston, C. W. and Gibson, R. B. A distilling apparatus and a procedure for the determination of alcohol in blood and urine. J. Lab. Clin. Med. 26:399. 1940.

59. Jolliffe, N. The influence of alcohol on the adequacy of the B vitamins in the American diet. Quart. J. Studies Alc. 1:74. 1940.

60. Jolliffe, N. and Wortes, H. Encephalopathia alcoholia. An evaluation of vitamin therapy. Am. J. Psychiat. 98:340. 1941.

61. Jungmickel, G. Technische Erleichterung bei der Widmarkschen Methode zur Alkoholbestimmung im Blut. Deut. Z. ges. gerichtl. Med. 32:537. 1940. Original not seen. Abstracted in Chem. Zentr. 111, I:3433. 1940.

62. Kluge, H. Eine spezifische Methode zur Blutalkoholbestimmung. Z. Untersuch. Lebensm. 78:449. 1939.

63. Koch, F, C. and McMeekin, T. L. A new direct nesslerization micro-Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. J. Am. Chem. Soc. 42:2066. 1924.

64. Künkele, F. Zur Verteilung des Alkohols im Organismus. Deut. Z. ges. gerichtl. Med. 31:253. 1939. Original not seen. Abstracted in Chem. Zentr. 110, II:1320. 1939.

65. Le Breton, E. Signification physiologique de l'oxydation de l'alcool éthylique dans l'organisme. Lons-le-Saunier, Maurice Declume, 1936. Thesis, Paris. See also: Compt. rend. Soc. biol. 122:565. 1936. Ann. physiol. physicochim biol. 12:169, 302, 805. 1936, and Bull. Soc. chim. biol. 19:17. 1937.

66. Levine, H. and Bodansky, M. A simple and accurate method for the determination of alcohol in body fluids. Am. J. Clin. Path., Tech. Suppl. 4:103. 1940.

67. Levy, J. Contribution à l'étude de l'accoutumance expérimentale aux poisons. II Alcoolisme expérimental. Fixation de l'alcool sur les tissus de l'organisme accoutumé a cette substance. Bull. Soc. chim. biol. 17:27. 1935.

68. Liljestrand, G. La distribution de l'alcool éthylique dans l'organisme et sa signification. Scientia 63:84. 1938.

69. Loewy, A. and von Der Heide, R. Über die Aufnahme des Äthylalkohols durch die Atmung. Biochem. Z. 86:125. 1918.

70. Lolli, G. and Greenberg, L. A. The effect of insulin on the rate of disappearance of alcohol from the stomach. Quart. J. Studies Alc. 3:92. 1942.

71. Mellanby, E. Alcohol: its absorption into and disappearance from the blood under different conditions. Brit. Med. Res. Council, Special report Ser. No. 31. 1919.

72. Miles, W. R. The comparative concentrations of alcohol in human blood and urine at intervals after ingestion. J. Pharmacol. 20:265. 1922.

73. Miller, F. E. and Getchell, R. W. Aspects of the blood alcohol tests to determine intoxication. Proc. Iowa Acad. Sci. 46:211. 1937.

74. Mirsky, I. A. and Nelson, N. The role of the liver in ethyl alcohol oxidation. Am. J. Physiol. 126:587. 1939.

75. Mirsky, I. A. and Nelson, N. The influence of the pancreas and the liver on the oxidation of ethyl alcohol. Am. J. Physiol. 127:308. 1939.

76. Mirsky, I. A., Piker, P., Rosenbaum, M. and Lederer, H. Adaptation of the central nervous system to varying concentrations of alcohol in the blood. Quart. J. Studies Alc. 2:35. 1941.

77. Mitchell, H. H. The food value of ethyl alcohol. J. Nutrit. 10:311. 1935.

78. Mitchell, H. H. and Curzon, E. G. The food value of ethyl alcohol. Quart. J. Studies Alc. 1:227. 1940.

79. Newcomer, H. S. A new optical instrument for the determination of hemoglobin, J. Biol. Chem. 55:569. 1923.

80. Newman, H. The determination of ethyl alcohol in body fluids. J. Pharmacol. and Exper. Therap. 56:278. 1936.

81. Newman, H. Acquired tolerance to ethyl alcohol. Quart. J. Studies Alc. 2:453. 1941.

82. Newman, H. W. and Lehman, A. J. Nature of acquired tolerance to alcohol. J. Pharmacol. 62:301. 1938.

83. Newman, H. W., Lehman, A. J. and Cutting, W. C. Effect of dosage on the rate of disappearance of alcohol from the blood stream. J. Pharmacol. 61:58. 1937.

84. Nicloux, M. Recherches sur l'alcool éthylique. Bull. Soc. chim. biol. 13:857. 1931.

85. Pringsheim, J. Chemische Untersuchung über das Wesen der Alkoholtoleranz. Biochem. Z. 12:143. 1908.

86. Rapin, A. Méthode de précision pour le dosage pondéral de petites quantités d'alcool en toxicologie par le mélange sulfo-chromique. Helv. chim. Acta. 22:72. 1939.

87. Research Council on Problems of Alcohol, Activities of. Reported quarterly in the Quart. J. Studies Alc.

88. Richter, C. P. and Campbell, K. H. Alcohol taste thresholds and concentrations of solution preferred by rats. Science. 91:507. 1940.

89. Schoen, F. Fehler bei Abnahme und Einsendung von Blutproben zur Blutalkoholbestimmung. Wien. Klin. Wschr. 53:724. 1940.

90. Selesnick, S. Alcohol intoxication, Its diagnosis and medicolegal implications. J. Am. Med. Assoc. 110:775. 1938.

91. Sheftel, A. G. A simple colorimetric method for determination of alcohol concentration in urine and blood. J. Lab. Clin. Med. 23:534. 1938.

92. Southgate, H. W. The effect of alcohol, under varying conditions of diet, on man and animals, with some observations on the fate of alcohol in the body. Biochem. J. 19:737. 1925.

93. Stempel, B. Vereinfachung der blutalkoholbestimmung nach Widmark. Pharm. Zentralhalle. 81:36. 1940.

94. Swim, J. B., McCowley, E. L. and Leake, C. D. Chemical tests for alcoholic intoxication. J. Am. Med. Assoc. 114:1098. 1940.

95. Tennent, D. M. Factors influencing the effects of alcohol on blood sugar and liver glycogen. Quart. J. Studies Alc. 2:265. 1941.

96. Tests for alcoholic intoxication. (Editorial). J. Am. Med. Assoc. 114:415. 1940.

97. Vanselow, A. P. Preparation of Nessler's reagent. J. Ind. Eng. Chem., Anal. Ed. 12:516. 1940.

98. Vollmering, J. Die verteilung des alkohols im organismus. Diss. Giessen. 1912.

99. Westerfield, W. W., Stotz, E. and Berg, R. The role of pyruvate in the metabolism of alcohol. Fed. Proc. (II). 1:140. 1942.

100. Widmark, E. M. P. Eine mikromethode zur bestimmung von äthylalkohol im blut. Biochem. Z. 131:473. 1922.

101. Widmark, E. M. P. Les lois cardinales de la distribution et du métabolisme de l'alcool éthylique dans l'organisme humain. Lund, C. W. K. Gleerup. 1930.

102. Widmark, E. M. P. Verteilung und Umwandlung des Äthylalkohols im Organismus des Hundes. Biochem. Z. 267:128. 1933.

103. Widmark, E. M. P. Über die Einwirkung von Aminosäuren auf den Alkoholgehalt des Blutes. Biochem. Z. 265:237. 1933.

104. Winnick, T. Determination of ethyl alcohol by micro diffusion. Ind. Eng. Chem., Anal. Ed. 14:523. 1942.

105. Wright, A. W. General pathology and some special complications of alcoholism. Arch. Path. 32:670. 1941.

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