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METABOLISM OF ETHYL ALCOHOL IN RATS EXPOSED TO COLD ENVIRONMENT

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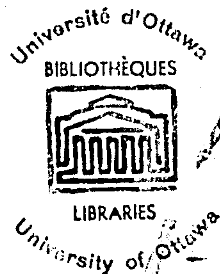
Submitted in partial fulfilment for the degree of
Doctor of Philosophy

1967

Candidate

April 7, 1967
Date

Supervisor



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ABSTRACT

The study of the metabolism of ethyl alcohol in rats exposed to a cold environment was investigated by means of uniformly ^{14}C-labeled ethyl alcohol.

In rats exposed to cold for five days, the rate of metabolism of alcohol was accelerated, in contrast to their controls kept at room temperature. Moreover, the rate of absorption of alcohol from the site of its administration and the rate of its removal from the blood was greater in cold-exposed animals than in rats maintained at room temperature. The observed increase in the rate of metabolism of alcohol in cold-exposed rats, however, was not proportional to the rate of increase in the general metabolism.

In cold-acclimated rats, whether or not pre-treated with alcohol, cold exposure resulted in an increased rate of alcohol metabolism. In cold-acclimated alcohol pre-treated rats, the increase in the rate of alcohol metabolism was proportional to the increase in overall metabolism.

The data also indicate that the rate of alcohol metabolism is essentially independent when given in various doses. However, a dose dependence on the ratio of oxidation of alcohol to the total foodstuff utilization was demonstrated.

No detectable changes in the alcohol oxidation rate were caused by partial hepatectomy.

A single dose of an adrenergic blocking agent, phenoxybenzamine, immediately prior to the introduction of

alcohol, produced a decrease in the oxidation rate of alcohol in rats exposed to cold. This reduction was more pronounced when cold-exposed rats were pre-treated with phenoxybenzamine from the onset of cold exposure, i.e. for five days prior to alcohol administration.

The positive influence of the thyroid gland on the metabolic rate of alcohol in animals exposed to cold was demonstrated by blocking hormone production with potassium perchlorate during the cold conditioning period.

STATEMENT OF THE PROBLEM

While the main features of the metabolic pathways of the breakdown of alcohol in the body have been known for some time, the picture is by no means complete. The sequences of its metabolism have been the subject of extensive investigations not only for its inherent interest to physiology and biochemistry but also because of the social, forensic and clinical consequences of the widespread consumption of alcoholic beverages by humans.

Despite a considerable amount of experimentation, in which various aspects of alcohol metabolism have been extensively described in the literature, few investigations have been made with respect to alcohol metabolism in animals exposed to a cold environment. Information was, therefore, sought as to the effect of short (5 days) and long (37-45 days) periods of cold exposure on the metabolism of alcohol in rats. In addition, insights were sought on the role of the catecholamines, the thyroid and the regenerating hepatic tissues (all of which are known to be involved in alcohol metabolism as well as in the overall metabolism of cold-exposed homeotherms) in relation to correlative changes of alcohol metabolism in rats exposed to cold. In this way it was hoped that information might be obtained on the metabolism of alcohol in the cold.

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INTRODUCTION

A-Intermediary Metabolism

Since the intermediary metabolism of ethyl alcohol has been extensively reviewed recently by Westerfeld (103), Westerfeld and Schulman (104), Lieber and Davidson (58), and Kalant (43), only a brief report on its breakdown will be given here. When administered orally to animals, most of the alcohol is readily absorbed from the stomach, the remainder from the intestinal tract. When given by other routes, it is absorbed rapidly from the site of administration. From two to ten per cent of the absorbed dose is eliminated by the kidneys or lungs, the remainder is oxidized in the body. Alcohol is initially oxidized to acetaldehyde (6) by alcohol dehydrogenase (92). This occurs almost entirely in the liver. Hepatic catalase, which is capable of taking part in the oxidation system of alcohol *in vitro* is considered to be without metabolic significance in the organism (4, 46, 47). Acetaldehyde is further metabolized in the liver or transported to other tissues where it is converted to a free acetate (83) via acetyl coenzyme A. The acetate moiety of the acetyl coenzyme A is then either oxidized to carbon dioxide and water, converted to fatty acids and ketone bodies, or incorporated into newly synthesized proteins, carbohydrates, or other biologically important materials (104).

B-The Effect of Temperature

It has been known for some time that environmental

temperature can influence the actions of drugs and chemicals in homeotherms. There have been, however, very few investigations concerning the relationship between environmental temperature and alcohol metabolism, and only a few reports have been made on the relationship between the body temperature and the metabolism of alcohol. Nicloux (71), Dybing (19) and Fuhrman (25), experimenting with frogs, rats, and rabbits respectively, reported an increase in the rate of metabolism of alcohol with an increase in body temperature. It would, however, appear that the variation in body temperature must be quite marked to exert a noticeable effect, since Le Breton (55) with rats and Ewing (20) with dogs found no change over a 4°C rise in body temperature. More recently, Forbes and Duncan (23) reported that the rate of alcohol utilization, as measured by its disappearance from the blood stream, was greater in rats exposed to a temperature of 2°C to 5°C, than in rats kept at room temperature. Keplinger, et al (45), examining the effect of environmental temperature on toxicity, found a much higher LD_{50} for alcohol in rats exposed to cold than in those kept at room temperature.

C-Prolonged Intake of Alcohol

Almost all the evidence obtained from earlier studies indicated that prolonged intake of alcohol does not lead to any adaptive increase in the rate at which alcohol can be metabolized in the body (43). Several workers (4, 46, 47, 104) have continued to investigate the possibility that in normal humans, liver catalase might serve as part of an auxiliary

enzyme system for the oxidation of alcohol to acetaldehyde, during continuous alcohol consumption. Evidence, however, was inconclusive. Recent work of Von Wartburg and Röthlisberger (90) suggested that catalase may play a much greater role in the metabolism of alcohol in alcoholics. The latter authors observed that long term administration of alcohol to rats resulted in increased liver catalase activity, whereas hepatic alcohol dehydrogenase was unaffected. Similar findings were reported by Trémolières and Carré (89) in humans with alcoholic cirrhosis. In vitro studies of liver homogenates and isolated hepatic cells from ethanol-treated (24 weeks) and untreated rats Dajani, and Orten (11) demonstrated that ethanol is better utilized by the liver of the treated rats than by the untreated controls. On the other hand, Segovia-Riquelme, et al (80, 81) did not find any significant difference in the rate of conversion to $^{14}CO_2$ of ethanol labeled in the first or second position between rats of the "drinker" and "non-drinker" strains.

D-Dose-Response Relationships

An important aspect of alcohol metabolism related to is rate of oxidation following various dose levels. In an extensive review on alcohol metabolism, Jacobsen (42) quoted thirteen papers on this subject. In seven of these it was concluded that the rate of alcohol metabolism is essentially independent of the dose of alcohol administered; six concluded that the rates diminished with increased dose. Six species of animals were utilized in these studies. The rate of alcohol metabolism was determined (a) by the shape of the curve of alco-

holemia versus time after administering a single dose of alcohol, (b) by the change in blood alcohol concentration during the constant infusion of alcohol, and (c) by complete body analysis for alcohol at various intervals following alcohol administration. Jacobsen (4[illegible]), in spite of the conflicting evidence, agreed with the majority of investigators that the rate of alcohol metabolism is practically independent of the amount of alcohol administered. He stated: "The rate of oxidation of alcohol in the organism is somewhat increased with increasing concentration of alcohol. Within the concentrations possible in the living organism this increase is so small that the elimination* curve of alcohol generally follows a straight line and for all forensic purposes no error is made if we assume a rectilinear elimination for alcohol."

More recently, a number of studies conducted with radioactive ethanol have provided evidence supporting the view that dose and rate of metabolism are not independent. Vitale, _et al_ (6) found in rats that after a one hour lag an optimum oxidation of alcohol was observed in the second and third hours with the rates of 330 to 400 mgm of alcohol per kgm of body weight per hour at doses of 2.0 to 2.5 gm of alcohol per kgm of body weight. Similar values were found by Segovia-Riquelme, _et al_ (81), when 2 gm per kgm of body weight of alcohol were administered. Conversely, Marshall and Owens (61) found that the rate of metabolism of ethanol in mice is appreciably greater in the first hour than during subsequent hourly intervals and concluded that, "The rate of oxidation of ethanol may be twice

*removal of alcohol from blood

as great in the first hour as in subsequent hours". The results of Kinard, et al (46, 47) do not support Marshall and Owens findings since they found no significant acceleration of metabolism in the mouse during the first hour following alcohol administration. However, Forney, et al (24) confirmed Marshall's conclusions, but in their study the early rapid rate of metabolism occurred during the first 30 minutes after injection of ethanol.

E-Role of the Liver

At present there can be little doubt that the liver is the major site of ethyl alcohol metabolism, and that the other tissues of the body play only an insignificant role. While early studies with ^{14}C labeled alcohol had indicated that small amounts of alcohol might be metabolized by slices of kidney, lung, and possibly other tissues in vitro (63), the bulk of evidence has always favored the view that the greatest proportion of alcohol oxication occurs in the liver. Liver slices and liver brei can oxidize alcohol in vitro (42). Isolated livers of dogs (22), cats (59) or rabbits (33) perfused with blood containing alcohol, remove the alcohol from the blood at a rate ranging from 1/2 to 5/6 of the rate expected in the whole organism. According to Clark, et al (9) eviscerated animals metabolize alcohol very slowly. Leomis (57) observed in hepatectomized dogs that very little alcohol had been metabolized. In partially hepatectomized animals the rate of metabolism of ethanol decreased, as more liver tissue was removed, to almost nil, when the animals were totally hepatectomized (64, 65). These and similar

results explain the fact that when the liver is damaged by arsenic, chloroform, phosphorus or antimony, the capacity of the organism to oxidize alcohol is lowered (42).

DesMarais (14), Hannon (34), Weiss and Moss (100-102), You and Sellers (110) and several other workers have shown that cold exposure in rats resulted in an increased oxidative activity of the liver. Weiss and Moss (102) reported that the liver, an organ which has one of the lowest metabolic rates among several tissues, showed the greatest metabolic increase in response to cold. The latter authors also have shown that the metabolic rate of liver slices in partially hepatectomized rats increased 20 per cent when animals are kept at room temperature and slightly over twice this amount when exposed to a cold environment.

F-Role of Catecholamines

It seems well established that production and secretion of catecholamines are increased in animals exposed to cold, an aspect of cold-acclimation which was extensively reviewed by Leduc (56). The extent to which catecholamines are involved in the regulation of chemical heat production in animals exposed to cold was reviewed by Hart (35).

Leduc (56) used the adrenergic blocking agent, phenoxybenzamine, to demonstrate the importance of catecholamines in the defense against cold. In his experiments cold-acclimated rats could not withstand cold exposure after blockade of the physiological effects of catecholamines, however, rats kept at room

temperature did not become hypothermic and did not show any visible shivering after phenoxybenzamine administration. This suggests that the block was not complete in warm-acclimated animals and that a sufficient amount of catecholamines can still reach the receptors and exert their actions to maintain a normal body temperature under these conditions. Moreover, according to this author, upon treatment with phenoxybenzamine, cold-acclimated rats survived cold exposure for a longer time than warm-acclimated ones. The longer survival-time of cold-acclimated rats treated with phenoxybenzamine was related to the increased sensitivity of these rats to adrenaline and noradrenaline, brought about by acclimation to cold. According to Holzbauer and Vogt (40), the tissues become hypersensitive to catecholamines after phenoxybenzamine administration. Moore (66) has suggested that in rats the calorigenic effects of adrenaline and noradrenaline were enhanced after pre-treatment with phenoxybenzamine.

These experiments do not yield any information concerning the respective role of adrenaline and noradrenaline, since the blocking effects of phenoxybenzamine are not specific for one or the other amine. It is also difficult to attribute the effect of catecholamines specifically to their vasoconstriction or metabolic action because phenoxybenzamine inhibits the blood pressure elevation as well as the hyperglycemia induced by adrenaline (36).

Hsienh, <u>et al</u> (41) have shown that, in cold-acclimated curarized rats, another adrenergic blocking agent, piperoxane, prevented the increase in oxygen consumption normally observed

upon exposure to cold, thus suggesting that catecholamines act through their effects on metabolism.

Recent studies on the relationship between ethyl alcohol and various organs with an autonomic nerve supply provide strong evidence that alcohol administration increases the secretion of adrenaline and noradrenaline from the adrenal medulla in men and in animals. In dogs with severe alcohol intoxication after doses of 6.4 to 12 gm/kgm, the adrenal catecholamine content was reduced and the urinary excretion of adrenaline and noradrenaline increased (48, 50-52). In other reports (31, 49), similar results were obtained when the doses of alcohol were reduced to 3.2 gm/kgm. Perman (74) demonstrated that, in cats adrenaline and noradrenaline concentrations in blood were increased after alcohol infusions. The effects of alcohol administration upon increased urinary adrenaline excretion in men have also been shown by Perman (73, 76). Klingman and Goodall (50) reported that alcohol similarly produced, in dogs, increased urinary elimination of adrenaline and noradrenaline. In rats, Wartburg, et al (98, 99) noted identical results following administration of a single dose of alcohol. However, when rats were kept on alcohol for a long period of time. Perman (75, 76) reported a decrease in urinary catecholamines excretion. Gursey and Olson (30) have reported that the level of noradrenaline and serotonine fell to about 50 per cent in the brain stem of rabbits after intravenous administration of ethanol. These results are at variance with those of Haggendal and Linquist (32), who showed that intravenous infusion of ethanol caused no significant changes in levels of noradrenaline, dopamine, and serotonine in rabbit

and mice brains.

G-Role of the Thyroid

Evidence has accumulated over a number of years concerning the role of the thyroid gland in the complex adaptive responses of homeotherms to cold environment (8, 14, 15, 82, 84). In rats these responses are associated with morphological and physiological changes in the thyroid gland. Most studies concerning thyroid function, based either on morphological evidence (3, 86, 88, 90, 91, 93) or on a variety of functional criteria (5, 10, 12, 54, 87, 106) show that at various periods of acute or chronic exposure to cold, the thyroid gland releases supranormal amounts of thyroid hormones into the circulation.

Several inorganic anions have been found to interfere with the normal function of the thyroid gland (85, 107, 108), the perchlorate and thiocyanate ions being outstanding in this group. The effect of perchlorate ions suggests a competition for some site in the gland (108). This assumption is based on the fact that perchlorate ions concentrate in the gland (2). The perchlorate ion is a competitive inhibitor of iodide for the "trapping" sites within the thyroid gland. It was shown by Anbar, _et al_ (2) that perchlorate ions do not undergo metabolization, thus excluding the possibility of their involvement in some enzymatic redox system. According to Anbar, _et al_ (1), the competition between perchlorate and iodide ions may be due to their similar monovalency and size. Wyngaarden, _et al_ (108) showed that perchlorate proved to be a very effective goitrogen, leading to marked hypertrophia, to a great increase in vascularity

of thyroid tissue, and to profound reductions of both soluble and precipitable iodide. As reported by Anbar, et al (2), it is apparent that rats tolerate large doses of perchlorate. In both animals and humans perchlorate is remarkable nontoxic, and inhibition of thyroid function can be achieved without producing the central nervous system depression which is characteristic of thiocyanate.

Several earlier workers have attempted, unsuccessfully, to increase the rate of alcohol metabolism by the administration of thyroxine or other agents which raise the general metabolic rate (43). Portet (77) in his recent work reported similar findings. He confirmed that pre-treatment of chick embryos with thyroxine failed to modify either the relative or absolute rate of alcohol metabolism per unit of body weight. However, Rawson, et al (78) claimed that a single dose of triiodothyronine produces a rapid return to complete sobriety of human subjects from alcoholic coma. Goldberg, et al (29) reported that triiodothyronine when given intravenously to an intoxicated human produces a highly significant increase in the rate of disappearance of alcohol from the blood. In contrast, Newman and Smith (69, 70) could not find any effect of triiodothyronine upon the rate of alcohol metabolism in dogs, and Kalant, et al (44) reported similar findings in humans.

Due to the numerous publications describing various facets of alcohol, particularly its metabolism in the animal body, this review has been necessarily restricted to those aspects pertinent to the present study.

EXPERIMENTAL PROCEDURE COMMON TO ALL EXPERIMENTS

Unless otherwise stated, male albino rats of the Sprague-Dawley strain weighing from 190 to 210 gm on day zero were used. They were individually housed in all wire cages, fed Purina Laboratory Chow and water *ad libitum*, the food being removed during the metabolic study of radioactive alcohol.

Uniformly labeled ^{14}C ethyl alcohol obtained from Merck, Sharp and Dohme of Canada, Limited was diluted to 20 per cent v/v with inert alcohol so that 1 mM of alcohol contained 0.23 microcurie. Following radioactive alcohol administration, the animal was placed immediately in a metabolic chamber. The expired CO_2 was collected in 2N NaOH by means of the apparatus described by Mackenzie, *et al* (60) and the radioactive carbonate precipitated with barium chloride. The barium carbonate was collected on filter paper according to the technique of Henriques, *et al* (37), and its radioactivity measured with a Nuclear Chicago Model C-110-A counter, preset to 2560 counts per sample. Duplicate samples were analyzed. Correction factors determined with the same instrument were used to correct for self absorption. In order to determine the $^{14}C_2H_5OH$ activity as an internal standard for the counter, the aqueous solution of radioactive alcohol was submitted to wet combustion by a slightly modified method of Van Slyke, *et al* (94), using Van Slyke, *et al* (95) reagents and the barium ^{14}C-carbonate obtained measured for its activity as above.

The intraperitoneal route of alcohol administration and its concentration were chosen to minimize the irritation at

the site of injection (38), and to obtain a more rapid and uniform absorption (68).

Specific activity of $^{14}CO_2$ representing the ratio of ^{14}C activity per total amount of exhaled respiratory CO_2 was calculated as the number of counts per minute per millimol of CO_2.

Urine samples were collected on a schedule based on the duration of the experiment (stated for each experiment), and the alcohol concentration determined by means of a Beckman Model GC-2 gas-liquid chromatograph using the technique of Cadman and Johns (7), as modified by Rokerbie (79). The ^{14}C activity in urine was measured by means of a beta-liquid scintillation detector, Nuclear Chicago Model 8401, using the Gjone, et al (27) method.

Analysis of variance was performed by IBM computer, Model 650, according to the "R x 2 Tables" of Yates' (109) completed by the Student's t test and/or "The Multiple Range Test" of Duncan (18) and Kramer (53).

EXPERIMENT I

METABOLISM OF ALCOHOL IN RATS EXPOSED TO COLD ENVIRONMENT FOR A SHORT PERIOD OF TIME

INTRODUCTION

The following experiment was divided into two parts:

Part I was of a preliminary nature to determine the effect, if any, of cold on the oxidation rate of alcohol by measuring its rate of conversion into respiratory CO_2.

Part II is an extension of Part I, using additional parameters to determine the effect of cold on the metabolism of alcohol. In addition to respiratory CO_2, blood, urine and body fluid alcohol content were measured and their validity as a measure of the oxidation rate of alcohol compared to $^{14}CO_2$.

MATERIALS AND METHODS

a-Part I

190-210 gm rats were divided into two groups of ten. The first group was exposed to 2^oC for five days and the second group kept at room temperature ($20^o \pm 2^oC$). On the sixth day each animal was injected with 0.8 gm/kgm of radioactive alcohol (0.23 µC/mM of alcohol). Respiratory $^{14}CO_2$ was collected at intervals of 1, 2, 3, 4, 6, 9 and 24 hours following alcohol administration, and its activity measured as previously described (p. 11).

b-Part II

Rats weighing 100 ± 5 gms were divided into two groups of 30 and given 1.6 gm/kgm of radioactive alcohol (0.23 µC/mM

alcohol). The first group was exposed to 2°C for five days, the second kept at room temperature. On the sixth day each animal was injected with 1.6 gm/kgm of radioactive alcohol (0.23 μC/mM of alcohol). Expired $^{14}CO_2$ was collected 1/4, 1/2, 3/4, 1, 1^1/2, 2, 3, 4, 6, and 9 hours after injection. Once the first urine sample was obtained (after two hours in the control group and four hours in the cold-exposed group) samples were then taken at the same time intervals as those for respiratory $^{14}CO_2$. At each time interval three rats were removed from the metabolic chamber and bled by cardiac puncture. The animal was then killed and the abdominal organs washed thoroughly with 0.9 per cent NaCl solution in order to remove non-absorbed alcohol that had diffused from the site of injection. Each animal was then homogenized in a Waring Blendor containing 5 N KOH. The homogenate was diluted to half a liter with the 5 N KOH. Alcohol concentration in the blood, urine and the homogenate of the whole animal were determined by gas-liquid chromatography. Activity of the various samples was then determined as previously described (p. 11). Widmark's beta factor (105) which is defined as the rate of decrease of total body alcohol from the rate of fall in the level of blood alcohol, after storage equilibrium had been reached, was measured during the first three hours following alcohol injection.

RESULTS

I-Respiratory $^{14}CO_2$

A-Cumulative Recoveries

Part I: In Table 1-a (p. 19) and Figure 1-a (p. 20) the cumulative mean recoveries of expired carbon dioxide from metabolized alcohol are plotted against the time elapsed after alcohol injection for each group of rats. The differences in the rates of conversion of alcohol into respiratory CO_2 of the two groups of animals due to temperature are evident. The differences between the groups were significant at the one per cent level ($P<0.01$) at all times of sampling. Examination of the shapes of the curves showed that the greatest acceleration of alcohol metabolism took place between 0 and 2 hours.

Part II: Measurements of similar parameter in the rats used in Part II of the experiment, Table 1-c (p. 23), confirmed the above results. The data in Table 1-c are averages from three different rats at each collection period for each group as opposed to the results shown in Table 1-a and Figure 1-a which were obtained from the same animals throughout. Even under these conditions, when greater variability might be anticipated, the differences between the cumulative $^{14}CO_2$ recoveries in each group were highly significant ($P<0.01$) at each of the ten collection periods.

B-Specific Activity

Part I: In Table 1-b (p.21) and Figure 1-b (p. 22) the specific activity of respiratory $^{14}CO_2$ at each collection period after alcohol administration is plotted for each group of rats.

It can be seen that rats exposed to cold for five days always exhaled less radioactive carbon dioxide in proportion to the total quantity of expired carbon dioxide, following injection of radioactive alcohol, than did their room temperature controls. The differences in the $^{14}CO_2$ specific activities of the two groups of animals were highly significant ($P<0.01$) for every collection period after the first hour.

II-Absorption

The abdominal washings from rats kept at room temperature and sacrificed 15 minutes after alcohol administration contained 0.6 per cent of the alcohol injected. Only a very slight trace was detectable in cold-exposed rats because of the limitations of the method used. All subsequent samples gave negative results.

As indicated by the data in Table 1-d (p. 24), however, the washings exhibited detectable radioactivity after alcohol could no longer be detected by chromatography. Thus, after the injection of $^{14}C_2H_5OH$, radioactive carbon was present in the peritoneal cavity for 30 minutes in rats exposed to cold and for twice this time in rats kept at room temperature. After 30 minutes, the activity of the abdominal washings from rats kept at room temperature was significantly higher ($P<0.01$) than the activity in similar washings from cold-exposed rats.

Complete absorption of radioactive carbon from the site of injection occurred within 45 minutes in the cold group as compared to 90 minutes in the controls.

III-Widmark's Beta Factor (105)

The rate of decrease in the blood alcohol concentrations

(i.e. Widmark's beta factor) of rats kept at room temperature and of those exposed to cold are shown below.

Rats exposed to cold...........1.078 ± 0.032 mg of alcohol/ 100 ml of blood/min.

Rats kept at room temperature..0.944 ± 0.036 mg of alcohol/ 100 ml of blood/min.

t............2.749

P............<0.05

Alcohol was detected in blood by gas-liquid chromatography for three hours following injection in rats exposed to cold and for four hours in those kept at room temperature. The above results were calculated for a one to three hour time interval following injection. The rate of disappearance from the blood was significantly greater in the cold-exposed rats.

IV-Alcohol Concentration in Rat Bodies

The concentrations of alcohol and radioactive carbon found in the alkaline homogenates of the bodies of the rats, including the amounts in the blood but not in the urine or abdominal washings, are shown in Table 1-e (p. 25) and Figure 1-e (p. 26). Alcohol could not be detected in the bodies of cold-exposed rats after four hours, and in those kept at room temperature after six hours. Elimination of ^{14}C followed the same trend but at different rates, being faster in cold-exposed rats; however, radioactive carbon was still detectable in the bodies of both groups of animals nine hours after alcohol administration. The differences in the alcohol concentrations of the two groups were significant ($P<0.05$) at 1/2, 1 and 3 hours, and more so ($P<0.01$) at 1^{1}/2 and 2 hours. Similarly, the differences

in the amounts of radioactive carbon were significant ($P<0.05$) at 3/4 and 6 hours and highly significant at ($P<0.01$) at $1^1/2$, 2, 3, 4 and 9 hours. It should be noted that in both groups of animals, the elimination of alcohol and of ^{14}C was rapid and essentially linear during the first three hours after administration of $^{14}C_2H_5OH$.

V-Alcohol in Urine

The recovery of alcohol and ^{14}C from the urine obtained from rats used in Part II is summarized in Table 1-f (p. 27). No urine samples could be collected during the first two hours following injection of alcohol from rats kept at room temperature and for four hours in those exposed to cold. The latter excreted significantly less alcohol ($P<0.01$) in their urine, but the recovery of ^{14}C was only slightly lower and not significantly different from the amount found in the urine of the rats kept at room temperature.

Table 1-a

The amounts of expired $^{14}CO_2$ following administration of $^{14}C_2H_5OH$ to rats exposed to cold and to rats kept at room temperature. Results are expressed as cumulative mean recoveries of millimols of CO_2 from metabolized alcohol.

Time of CO_2 collection	Animals kept at room temperature	Animals exposed to cold	t*
1 hour	0.80 ± 0.06	1.38 ± 0.10	5.092
2 cum. hours	2.64 ± 0.18	4.00 ± 0.16	5.609
3 cum. hours	3.72 ± 0.08	4.96 ± 0.10	9.670
4 cum. hours	4.18 ± 0.10	5.26 ± 0.06	9.611
6 cum. hours	4.60 ± 0.10	5.62 ± 0.06	8.324
9 cum. hours	4.88 ± 0.12	5.88 ± 0.06	7.383
24 cum. hours	5.54 ± 0.08	6.12 ± 0.06	5.412

*$P<0.01$ in every instance.

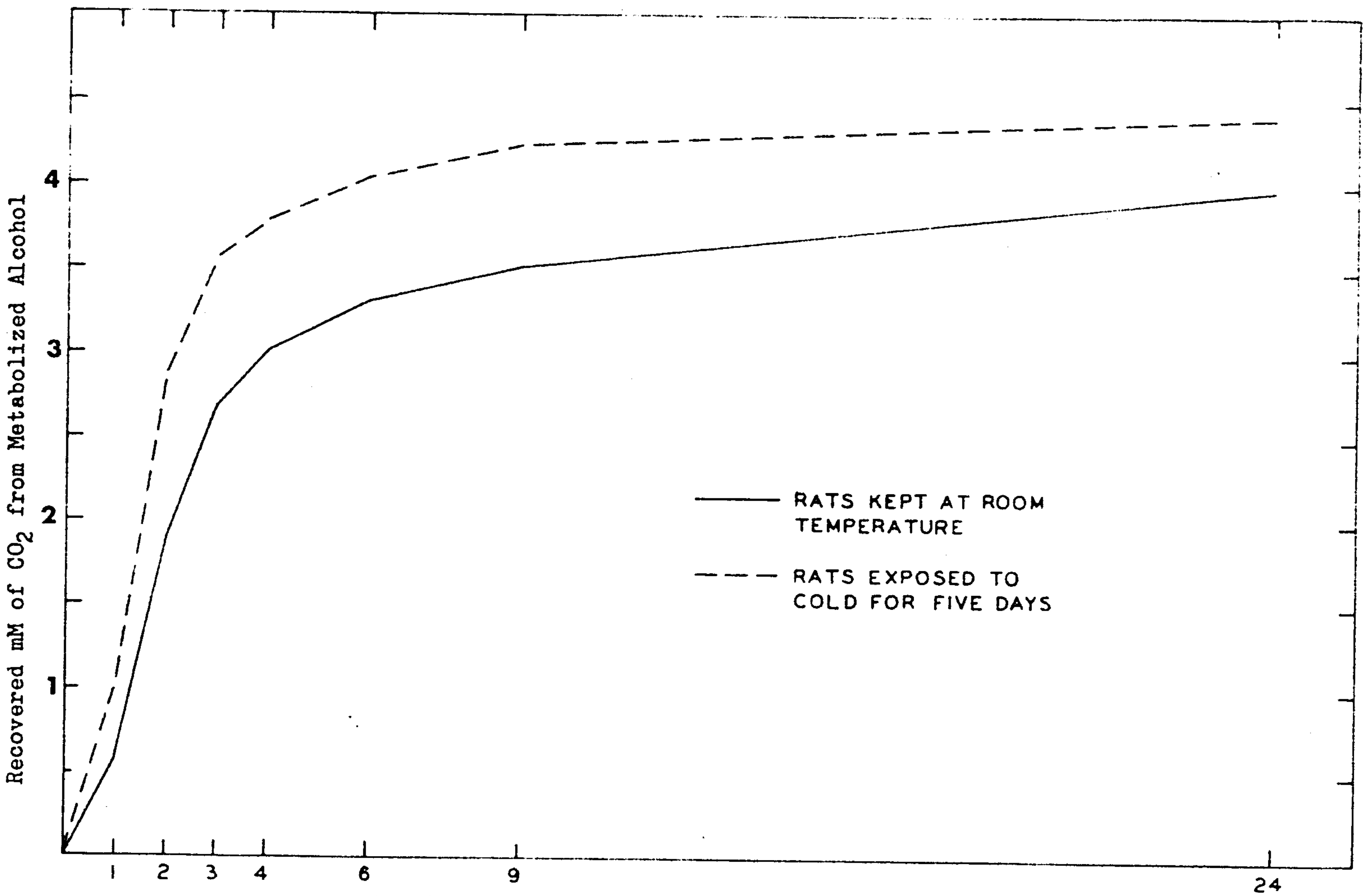

Figure 1 - a. Cumulative recoveries of mM of CO_2 from metabolized alcohol, administered to rats exposed to cold and to others kept at room temperature.

Table 1-b

The specific activity of $^{14}CO_2$ at different intervals following the injection of $^{14}C_2H_5OH$ to rats exposed to cold and to others kept at room temperature. Results are expressed as the number of counts per minute per millimol of expired CO_2.

Time of CO_2 collection	Animals kept at room temperature	Animals exposed to cold	t	P
1 hour	3641 ± 213	3087 ± 501	0.983	N.S.
2 hours	7571 ± 227	4190 ± 318	8.607	<0.01
3 hours	4674 ± 592	2269 ± 231	3.775	<0.01
4 hours	2149 ± 233	928 ± 108	4.736	<0.01
6 hours	1194 ± 114	440 ± 2	6.149	<0.01
9 hours	505 ± 4	156 ± 8	79.372	<0.01
24 hours	181 ± 10	41 ± 4	11.993	<0.01

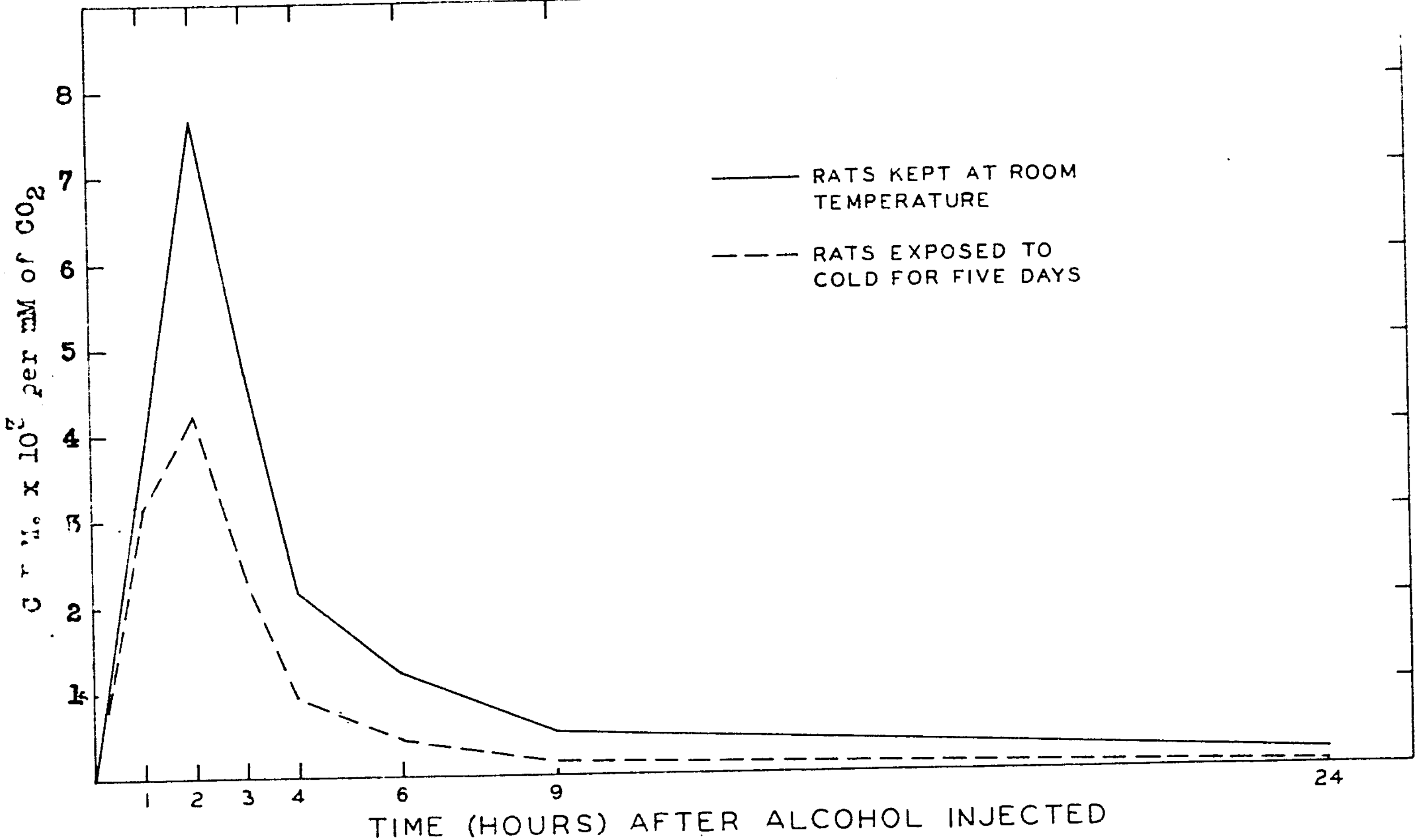

Figure 1 - b. The specific activity of $^{14}CO_2$ at different intervals following injection of radioactive alcohol to rats exposed to cold and to others kept at room temperature. Results are expressed as the number of counts per minute per millimol of expired CO_2.

Table 1-c

Cumulative recoveries of expired mM of CO_2 from metabolized alcohol.

Time from injection (hr.)	Animals kept at room temperature	Animals exposed to cold	t*
1/4	0.18 ± 0.02	0.38 ± 0.04	5.214
1/2	0.38 ± 0.04	0.94 ± 0.06	9.057
3/4	0.64 ± 0.06	1.00 ± 0.06	13.437
1	0.80 ± 0.04	1.58 ± 0.14	5.641
1 1/2	1.94 ± 0.04	2.72 ± 2.08	8.851
2	2.66 ± 0.02	4.00 ± 0.08	16.031
3	3.64 ± 0.16	5.14 ± 0.08	8.584
4	4.30 ± 0.12	5.18 ± 0.06	7.069
6	4.88 ± 0.12	5.78 ± 0.06	6.881
9	5.42 ± 0.12	6.08 ± 0.08	4.528

*$P<0.01$ in every instance.

Table 1-d

Radioactivity in abdominal washings from rats kept at room temperature and those exposed to cold. The results are the average amounts of ^{14}C recovered, expressed as a percentage of the amount administered.

Time after injection (hr.)	Animals kept at room temperature	Animals exposed to cold	t	P
1/4	1.79 ± 0.36	1.34 ± 0.26	1.009	N.S.*
1/2	1.81 ± 0.12	1.03 ± 0.05	6.059	<0.01
3/4	1.64 ± 0.18			
1	1.03 ± 0.08			

* N.S. = not significant

Table 1-e

Recovery of alcohol and ^{14}C from the bodies of rats exposed to cold and others kept at room temperature following $^{14}C_2H_5OH$ administration. Results are expressed as percentages of the dose injected.

Time from injection (hr.)	Alcohol			^{14}C		
	Room T.	Cold	t	Room T.	Cold	t
1/4	88.77 ± 1.97	88.95 ± 0.57	0.09	91.44 ± 1.23	93.35 ± 1.07	1.17
1/2	81.24 ± 0.54	73.10 ± 2.85	2.81*	82.83 ± 1.11	77.12 ± 2.54	2.06
3/4	74.68 ± 1.48	68.26 ± 3.53	1.68	77.61 ± 2.28	68.14 ± 1.64	3.36*
1	70.15 ± 1.55	64.36 ± 2.27	2.58*	72.52 ± 3.79	60.00 ± 4.14	2.23
$1^1/2$	56.85 ± 1.21	44.11 ± 2.31	4.87**	61.90 ± 1.58	47.49 ± 1.09	7.49**
2	42.91 ± 1.72	28.97 ± 0.89	7.19**	44.17 ± 2.02	31.75 ± 1.01	5.50**
3	20.93 ± 1.80	11.21 ± 0.96	3.99*	26.03 ± 2.27	15.14 ± 1.13	4.30**
4	10.77 ± 0.37	N.D.		17.09 ± 0.54	8.39 ± 1.03	7.48**
6	N.D.	N.D.		13.49 ± 1.70	6.59 ± 1.23	3.29*
9	N.D.	N.D.		8.72 ± 0.71	4.07 ± 0.67	4.76**

N.D. non-detectable; * $P<0.05$; ** $P<0.01$

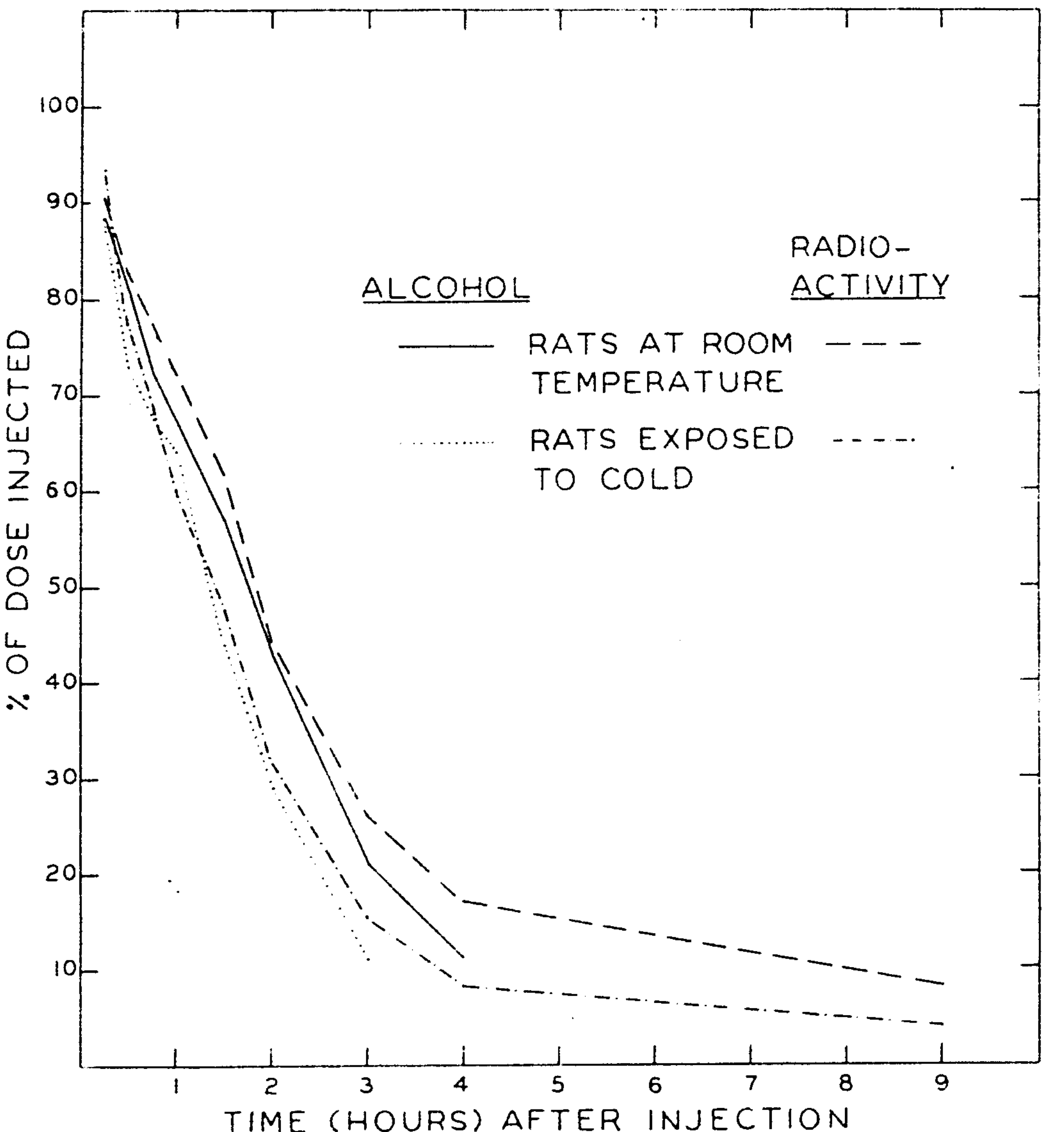

Figure 1-e. Decrease in alcohol and C^{14} concentrations in the bodies of rats exposed to cold and those kept at room temperature following $C_2^{14}H_5OH$ administration.

Table 1-f

Recovery of alcohol and radioactive carbon from the urine of rats exposed to cold and those kept at room temperature. Results expressed as percentages of the administered dose.

Time from injection (hr.)	Animals kept at room temperature		Animals exposed to cold		Student's t	
	Alcohol	^{14}C	Alcohol	^{14}C	Alcohol	^{14}C
2	0.59 ± 0.001	1.85 ± 0.45				
3	0.89 ± 0.030	2.61 ± 1.65				
4	1.79 ± 0.150	2.51 ± 0.64	0.42 ± 0.043	2.07 ± 0.05	8.766**	0.668
6	2.83 ± 0.140	4.12 ± 0.93	0.62 ± 0.064	2.92 ± 0.23	14.399**	1.641
9	3.25 ± 0.103	4.98 ± 0.94	1.38 ± 0.451	3.95 ± 0.32	12.687**	0.093

** $P < 0.01$

DISCUSSION

The results presented in Part I (given as cumulative $^{14}CO_2$ recovery) clearly demonstrated that in cold-exposed rats there is an increased rate of conversion of alcohol into respiratory CO_2 as compared to animals kept at room temperature. This enhancement was particularly pronounced within the first three hours following alcohol administration, i.e. during the period when most of the alcohol was metabolized. The peak of alcohol oxidation was not affected by environmental conditions and occurred during the second hour. In Part II, where smaller animals were utilized (since it was found that bigger than 100 gm animals could not be homogenized in a Waring Blendor) and where the dose of alcohol administered was double (only for the sake of more accuracy during the chemical analyses of alcohol) that used in Part I, an identical pattern of alcohol metabolism was observed. For the measurement of this parameter, the radioactivity of CO_2 was used as an index of the amount of alcohol metabolized.

Using the same index of measurement, Vitale, et al (96) reported that in rats kept at room temperature, the maximal recovery of expired $^{14}CO_2$ from labeled alcohol occurred during the second and third hours.

In most previous studies (42, 43) changes in blood alcohol concentration have been used as the measure of oxidation, in which only the oxidation of alcohol to acetaldehyde would be necessary to cause the disappearance of alcohol from blood. It seems unlikely, however, that the difference in extent of oxidation have affected the results obtained. The synthesis of other

metabolic products from alcohol or the accumulation of intermediate products must be minimal. Since we have measured in addition to $^{14}CO_2$ recovery, the disappearance of alcohol from blood as well as from total body fluids, we are in a position to compare these parameters.

The picture of alcoholemia observed in Part II followed an identical pattern to the conversion rate of alcohol into CO_2 seen in the same animals. Thus, it would seem safe to assume that for all practical purposes the method of measurement of respiratory $^{14}CO_2$ yields essentially the same results as the direct measurement of the disappearance of alcohol from the blood or from the total body.

Forney, _et al_ (24), employing a dose of ethanol of 4 gm/kgm, found a rapid rate of removal of alcohol from blood 30 minutes after injection and thereafter a slower almost constant rate. On the other hand, Fazekas (21) reported that a maximum peak was reached at 3 hours following administration of 2 gm/kgm of alcohol.

It is an established fact that exposure of homeotherms to a cold environment results in elevation of energy metabolism which leads to well-defined alterations in metabolic processes, the most important of which is the development of an enhanced capability to elevate metabolism and maintain it at a high level. The increased metabolic energy resulting from exposure of animals to cold leads one to conclude that increased oxidation of the proteins, carbohydrates or lipids present in the body must occur. Several reports have appeared which indicate that the increase is proportionately distributed over all metabolic processes (13). Dontcheff

and Fortet (17) reported that in rats kept at room temperature, administered alcohol is utilized in definite proportion to the foodstuff oxidized at the same time and that this proportion is independent of the dose of administered alcohol. In our experiments where two different yet relatively small doses of alcohol (0.8 and 1.6 gm/kgm) were administered, while the rate of alcohol oxidation increased in cold-exposed animals (Tables 1-a and 1-c), this increase was not proportional to the increase in general metabolism. Specific activity of respiratory $^{14}CO_2$ (Table 1-b and Figure 1-b), except for the hour following alcohol administration, was significantly (P<0.01) lower throughout the duration of the experiment in animals exposed to cold than in their controls kept at room temperature. In animals kept at room temperature ethyl alcohol provokes cutaneous vasodilation and sweating, leading to considerable increase in the rate of heat loss. In animals exposed to cold, this may not be entirely compensated for by increased heat production, because of the increased depressant or narcotic action of alcohol on the central nervous system.

EXPERIMENT II

METABOLISM OF ALCOHOL IN COLD-ACCLIMATED, ALCOHOL-PRETREATED RATS

In the previous experiment, it was observed that the rate of metabolism of alcohol is higher in rats exposed to cold for a short period of time, than in rats kept at room temperature. This increase, however, did not coincide with a general increase in metabolism due to cold exposure. It was thus decided to investigate the extent to which cold could influence the metabolism of alcohol in cold-acclimated rats as well as in rats which had received alcohol daily during the process of cold-acclimation.

MATERIALS AND METHODS

Group 1 was exposed to 2°C for 37-45 days.

Group 2 was exposed to 2°C for 37-45 days.

Half of the animals from each group received 0.8 gm/kgm of a 20 per cent v/v of non-radioactive alcohol daily by intraperitoneal injection.

Groups 3 and 4 served as room temperature controls for the above groups receiving otherwise identical treatment.

Following the exposure period, each animal received a single injection (0.8 gm/kgm) of radioactive alcohol. Expired $^{14}CO_2$ was collected at intervals of 1, 2, 3, 4, 6, 9 and 24 hours. A total 24 hour urine collection was made following radioactive alcohol administration. The metabolism of alcohol was determined by the previously described methods (p. 11).

RESULTS

I-Respiratory $^{14}CO_2$

A-Cumulative Recoveries

The rate of alcohol metabolism, as measured by means of cumulative recoveries of $^{14}CO_2$ at specific intervals, is shown in Table 2-a (p. 34) and Figure 2-a (p. 37). The respiratory output of radioactive carbon dioxide in cold-acclimated rats, whether or not the animals were given alcohol daily, were significantly different from their control groups kept at room temperature. The slope of recovery of $^{14}CO_2$ was nearly linear for the first three hours in cold-acclimated rats and for the first four hours in those maintained at room temperature. However, the cold groups eliminated more $^{14}CO_2$ in three hours than their counterparts did in four hours. Alcohol pre-treatment did not modify the rate of alcohol metabolism either in the cold or at room temperature.

B-Specific Activity

In Table 2-b (p. 38) and Figure 2-b (p. 41) the results are given in terms of the specific activity of respiratory $^{14}CO_2$ at each collection period. It is evident, from a study of Figure 2-b, that the animals exposed to cold exhaled less $^{14}CO_2$ in proportion to the total quantity of exhaled carbon dioxide, than those maintained at room temperature, confirming what was observed in the first experiment. The alcohol pretreated, cold-acclimated rats, however, have a pattern of excretion similar to that of the control groups for the first two hours, but thereafter the relative curve of alcohol combustion was identical to the curve of the other group of cold-acclimated, non-alcohol pretreated rats.

II-Urinary Excretion of Alcohol

Recovery of ^{14}C activity and the total alcohol in the urine collected for 24 hours, is given in Table 2-c (p. 42). Urine of warm-acclimated rats, whatever the treatment, contained more radioactive carbon than the urine of cold-acclimated animals. Alcohol was not found in any detectable quantity in the urine of cold-acclimated rats.

Table 2-a

Mean values of cumulative recoveries of exhaled CO_2 from metabolized alcohol.

Treatment	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
Room T.	0.81	2.63	3.71	4.18	4.59	4.87	5.54
Cold acclim.	1.24	3.44	4.33	4.64	4.99	5.22	5.81
Room T. and alcohol daily	0.65	2.20	3.10	3.95	4.48	4.81	5.32
Cold acclim. and alcohol daily	1.53	3.71	4.54	4.85	5.19	5.40	5.76

Table 2-a (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
A	1.813	0.076	0.507	0.001	0.101	0.140	0.640
B	15.524**	17.157**	13.123**	15.540**	12.367**	9.351**	4.527*
AB	1.791	1.549	2.145	1.642	0.994	0.617	0.257

A: Effect of treatment; B: Effect of temperature; AB: Interaction

* $P < 0.05$

** $P < 0.01$

Table 2-a (continued)

Averages and Student's t values of combined groups kept at room temperature and of groups exposed to cold, without regard to treatment.

	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
Room T.	0.71	2.37	3.34	4.0[illegible]	4.53	4.83	[illegible].40
Cold	1.39	3.58	4.43	4.75	5.09	5.31	5.78
Student's t	6.830**	7.677**	5.483**	6.622**	5.022**	3.908**	2.721*

* $P < 0.05$

** $P < 0.01$

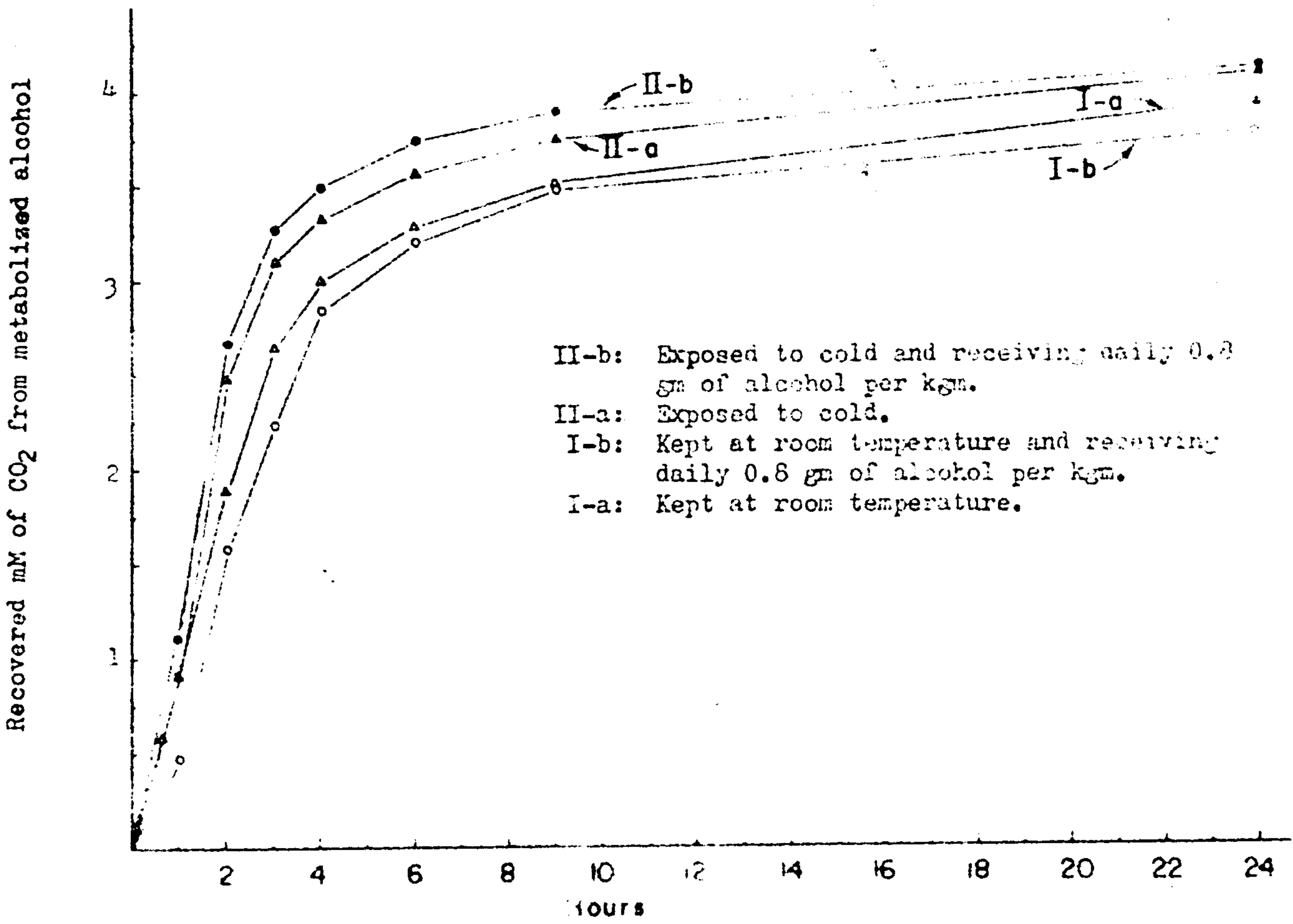

Figure 2-a. Cumulative recoveries of mM of CO_2 from metabolized alcohol.

Table 2-b

Mean values of $^{14}CO_2$ specific activity, represented as c.p.m. per mM of expired CO_2.

Treatment	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
Room T.	3641	7571	4804	2149	1194	505	181
Cold acclim.	3049	4680	1826	734	391	225	105
Room T. and alcohol daily	3039	6967	5872	3201	1656	572	22
Cold acclim. and alcohol daily	4682	6515	2445	1014	551	235	105

Table 2-b (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
A	2.072	1.327	1.359	2.535	2.029	0.309	0.626
B	2.104	9.782**	19.597**	18.468**	19.059**	20.516**	14.189**
AB	9.697**	5.210*	0.095	0.847	0.478	0.183	0.603

A: Effect of treatment; B: Effect of temperature; AB: Interaction

* $P < 0.05$

** $P < 0.01$

Table 2-b (continued)

Averages and Student's t values of combined groups kept at room temperature and of groups exposed to cold, with regard to treatments

	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
Room T.	3280	7226	5414	2749	1456	545	205
Cold	3862	5597	2135	874	472	231	103
Student's t	1.885	4.078**	8.658**	8.023**	8.213**	8.907**	7.085**

** $P < 0.01$

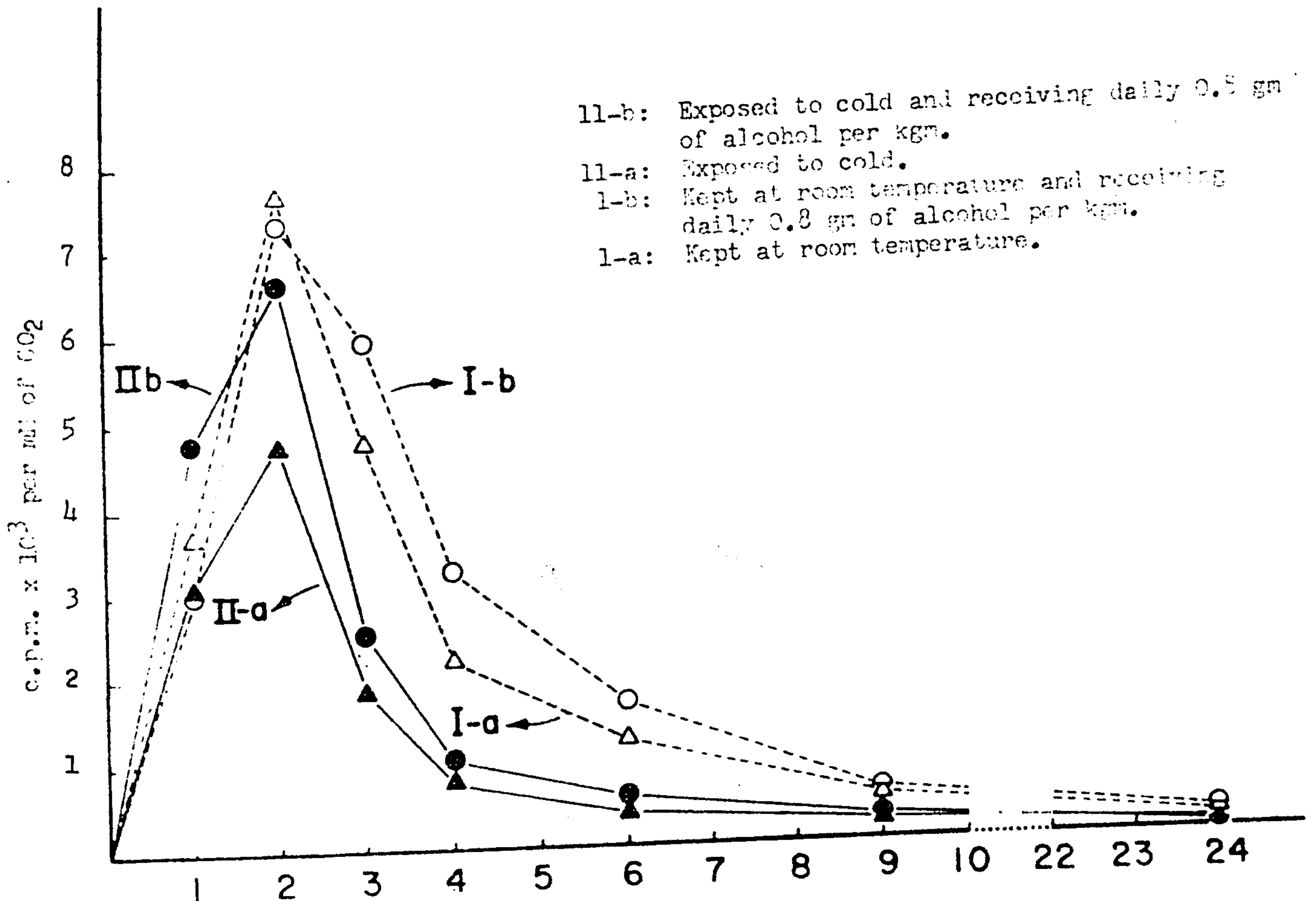

Figure 2 - b. The specific activity of $^{14}CO_2$ at different intervals following injection of radioactive alcohol to rats exposed to cold and others kept at room temperature. Results are expressed as the number of counts per minute per millimol of expired CO_2.

Table 2-c

Recovery of alcohol and ^{14}C in the urine collected for 24 hours and expressed as the percentages of $^{14}C_2H_5OH$ or inert alcohol administered.

Group of animals	Alcohol	^{14}C
Rats kept at room T.	1.07 ± 0.38	2.76
Rats kept at room T. and pre-treated with alcohol	0.58 ± 0.10	2.80
Rats exposed to cold		0.34
Rats exposed to cold and pre-treated with alcohol		0.59

Student's t = 1.268 for alcohol datta

F values from Yates' (109) Analysis of Variance for ^{14}C data

Source of variance	F value	P
Effect of treatment	0.075	N.S.
Effect of temperature	19.461	>0.01
Interaction	0.039	N.S.

Table 2-c (continued)

^{14}C Means and Student's t value of combined groups kept at room temperature and of groups exposed to cold, without regard to treatment

Room T.	Cold exposed	Student's t	P
2.79	0.46	8.087	>0.01

DISCUSSION

As in the previous experiment, cold exposure resulted in an increased rate of alcohol metabolism. Nevertheless, it would appear that the acclimation process is not responsible for this enhancement of alcohol metabolism, since in previous experiments where rats were exposed to cold environment for only short periods a similar increase in alcohol combustion was observed. As shown in Figure 2-a, there was little or no effect of ethanol pre-treatment on the apparent rate of its metabolism.

On the other hand, there appears to be some effect of alcohol pre-treatment on the ratio of respiratory CO_2 derived from ethanol versus total respiratory CO_2. As shown in Figure 2-b, Curve II-b shows that this relative ratio is elevated after alcohol pre-treatment at the second hour sample (compare Curve II-a).

Our experimental design gave a measure of the combustion of ethyl alcohol, that is, the over-all conversion of alcohol carbons to carbon dioxide as detected by the radioactivity of CO_2 formed. This procedure produced evidence of the rate of its oxidation but gave no information about the intermediate reactions. By measuring the specific activity of $^{14}CO_2$, however, the ratio of alcohol metabolism to general metabolism was determined.

Although we have no way to explain why in "alcoholic" cold-acclimated rats at the peak of alcohol metabolism the relative utilization of alcohol is comparable with that of the controls kept at room temperature (Figure 2-b, Curves I-a and II-b), this may be due to some change in the metabolic pathways of alcohol.

Myers (67) observed that following repeated intracranial administration of alcohol, rats exhibited pronounced preferences for ethanol. He suggested that intracranial infusion acted as a non-specific stressor, causing the rats to drink ethanol to relieve the stress and the chronic or direct alteration of the brain's biochemical "environment" can produce significant changes in later behavior. As discussed in the Introduction, Von Warburg and Pöthlinger (99) and Trémolière and Carré (89) suggested that in the human alcoholic, liver catalase may play a much greater role in the metabolism of alcohol. If it is assumed that catalase was involved in the increased relative metabolism of alcohol in cold-acclimated "alcoholics", there should be a similar effect in warm-acclimated "alcoholics". The latter group, however, has a metabolic pattern in all respects similar to that of the untreated controls. On the other hand, it should be borne in mind that cold-acclimated, alcohol pretreated rats were submitted to two distinct stresses, i.e., the daily administration of alcohol and the cold environment. Considering those two factors, we are inclined to assume that the relative increase in alcohol metabolism in alcohol-pretreated, cold-acclimated rats, in contrast to their untreated counterparts, was due to some enzymatic adaption of an unknown nature. Depression of basal metabolic rate as the result of pre-treatment would also produce the same effect.

EXPERIMENT III

DOSE-RESPONSE RELATIONSHIP TO ALCOHOL IN RATS EXPOSED TO COLD

In view of the foregoing results, it was of interest to compare the effect of different doses of ethanol on the rate of alcohol metabolism in rats exposed to cold with that of rats maintained at room temperature.

MATERIALS AND METHODS

Rats weighing between 190 and 210 grams were divided into six groups of no less than six animals per group. Groups 1, 2 and 3 were maintained at 2°C for five days prior to alcohol administration, while Groups 4, 5 and 6 were kept at room temperature.

On day six the following injections of radioactive ethanol were administered intraperitoneally:

a) Groups 1 and 4 - 0.8 gm/kgm,

b) Groups 2 and 5 - 2.4 gm/kgm,

c) Groups 3 and 6 - 4.0 gm/kgm.

Although the quantity of ethanol varied, the amount of ^{14}C-ethanol was the same, i.e., 0.8 microcurie per animal. Expired $^{14}CO_2$ and urine were collected at different time intervals for 24 hours.

The methods used for the recovery of $^{14}CO_2$, the alcohol and ^{14}C content of the urine are similar to those described previously.

The rates of metabolism were determined from the slopes

of the linear portion of the curves obtained by plotting the $\log_{10}$ respiratory $^{14}CO_2$ recovered *versus* time of sampling.

RESULTS

I-Respiratory $^{14}CO_2$

A-Cumulative Recoveries

Table 3-a (p. 51) and Figure 3-a (p. 54) illustrating the cumulative recoveries of CO_2 derived from metabolized alcohol, clearly show the independence of rate of conversion of alcohol to carbon dioxide of the dose of alcohol adminstered, although the initial metabolic rates of alcohol seemed to be somewhat lower at higher doses of alcohol. Similarly, there was no significant effect of environmental temperature on the rates of alcohol metabolism during the course of the experiment. The recovered $^{14}CO_2$ was plotted as an exponential function against time (Figure 3-a, p. 54) and gave nearly linear curves. The point at which the curves levelled off asymptotically was proportional to the dose administered, as expected. Thus, with the doses of alcohol of 0.8, 2.4 and 4 gm/kgm the curves were linear for 2, 6 and 9 hours, respectively. At 9 hours the difference between groups given 0.8 gm of alcohol per kgm and those given 2.4 and 4 gm/kgm was significant at the 5 per cent level. There was a highly significant difference ($P<0.01$) between all groups when considered over the full 24 hours period.

B-Specific Activity

The ratio of $^{14}CO_2$ derived from $^{14}C_2H_5OH$ to the total amount of CO_2 exhaled and originating from the total foodstuff metabolized during the same period, does not show the same pattern as above. The results are shown in Table 3-b (p. 55) and Figure 3-b (p. 58). The difference between cold-exposed groups and the control group occurred at the 0.8 gm/kgm level and is

similar to what was observed in the first experiment. The cold-exposed rats given 2.4 and 4 gm of alcohol per kgm had specific activity patterns practically identical to their control groups kept at oom temperature for the first six hours, after which the control groups exhibited significantly ($P<0.01$) higher values of $^{14}CO_2$ specific activity until the end of the experiment. On the other hand, when the values of the specific activity of $^{14}CO_2$ were compared at various dose levels the data clearly show that with the higher doses of alcohol a depression of general metabolism took place at both high and low temperature. Thus the specific activity of $^{14}CO_2$ obtained from 0.8 gm/kgm groups was smaller than that of 2.4 gm/kgm groups and the latter was smaller than that of the groups given 4 gm of alcohol per kgm of body weight. These differences were highly significant ($P<0.01$) throughout the duration of the experiment.

Rats receiving the 2.4 gm/kgm dose showed the evidence of muscular incoordination within a few minutes of injection; those given 4 gm/kgm were in a state of near collapse for a period of four to six hours. The behavioural findings could explain the above results. In animals given the 0.8 gm of alcohol per kgm no noticeable changes in behaviour were observed.

II-Urinary Excretion of Alcohol

The amount of unchanged alcohol eliminated via the urine was higher in the control group than in animals exposed to cold, at the 0.8 gm/kgm dose level. With the three and fivefold increase in the dose of alcohol, the percentage of alcohol eliminated via the urinary system was greater than at the 0.8 gm/kgm level. At the higher dose levels, temperature had no noticeable

effect (Table 3-c, p. 59).

Table 3-a

Cumulative recovery of expired CO_2 (in mM) from metabolized alcohol administered at doses of .8, 2.4, and 4 gm/kgm.

Treatment	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
0.8 gm/kgm, room T.	0.81	2.63	3.71	4.18	4.59	4.87	5.54
0.8 gm/kgm, cold	1.38	3.99	4.95	5.26	5.61	5.88	6.11
2.4 gm/kgm, room T.	0.43	1.57	3.32	5.25	10.14	15.37	18.17
2.4 gm/kgm, cold	0.77	2.98	5.44	8.23	12.88	16.27	17.72
4 gm/kgm, room T.	0.34	1.17	2.53	4.19	7.48	14.51	26.72
4 gm/kgm, cold	0.55	1.83	3.43	5.23	8.64	14.89	24.58

Table 3-a (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data.

Source of Variance	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
A	2.59	2.15	1.50	1.31	1.12	1.02	0.99
B	2.36	1.82	1.19	1.14	1.82	3.86*	9.21**
AB	0.99	0.98	1.10	1.14	0.09	1.01	1.01

A: Effect of temperature; B: Effect of treatment; AB: Interaction

*$P<0.05$

**$P<0.01$

Table 3-a (continued)

Duncan - Kramer (18, 53) test of significance to determine the effect of treatment by combining groups kept under different environmental conditions but receiving various treatment.

9 hrs.	Group	0.8 gm	4.0 gm	2.4 gm
	Mean	5.37	14.70	15.81
24 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	5.82	17.94	25.65

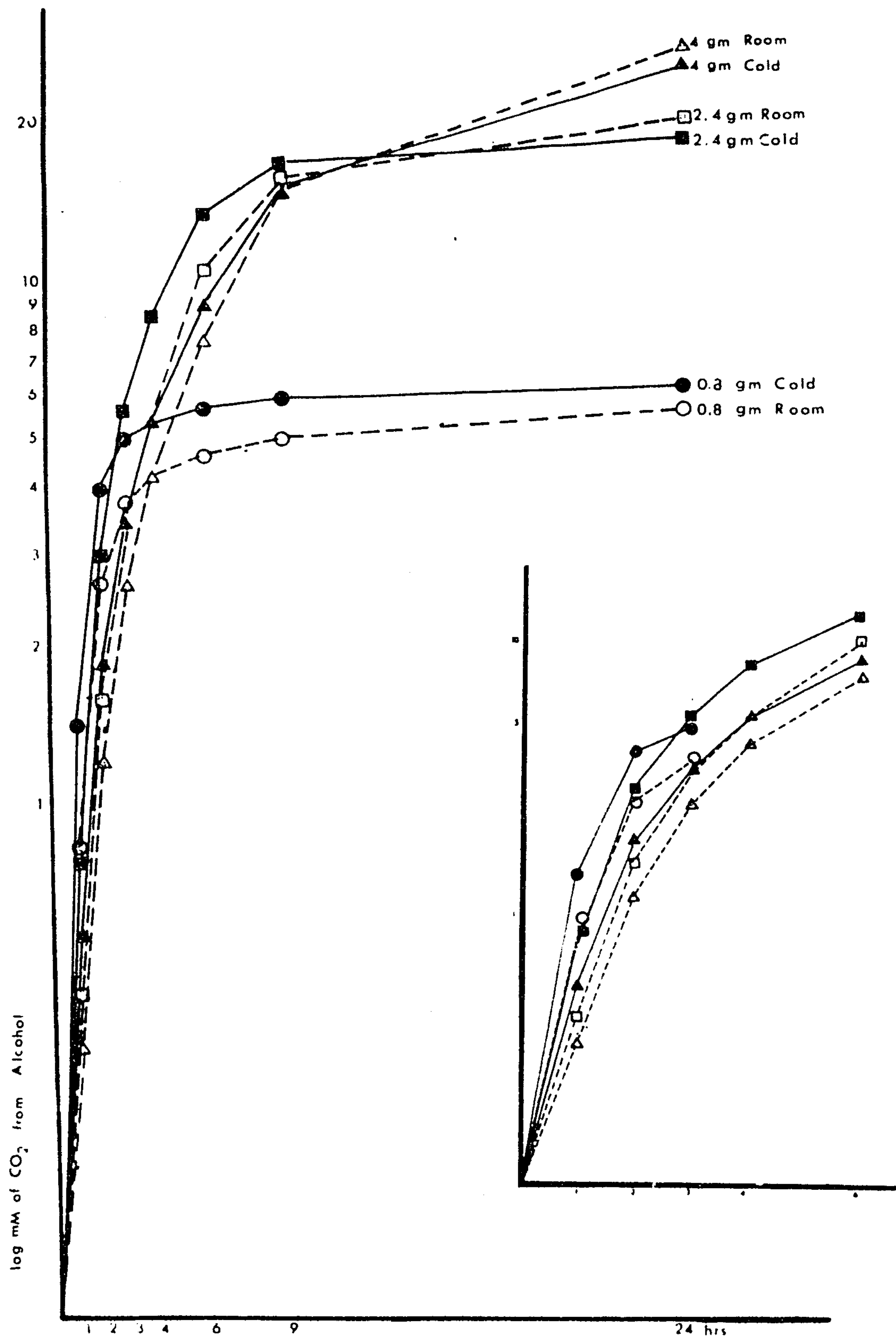

Figure 3-a. Rates of ethanol oxidation as a function of dose versus rates exponential as hrs^{-1}. Results are given in terms of recovered mM of CO_2 from metabolized alcohol. Expanded linear part is given on the right side.

Table 3-b

Mean values of $^{14}CO_2$ specific activity, represented as c.p.m. x 10^2 x dilution factor per mM of exhaled carbon dioxide.

Treatment	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
0.8 gm/kgm, room T.	36.41	75.71	46.74	21.49	11.94	5.05	1.81
0.8 gm/kgm, cold	30.87	41.90	22.90	9.28	4.40	1.56	0.41
2.4 gm/kgm, room T.	47.84	125.29	170.29	199.42	280.19	160.76	42.45
2.4 gm/kgm, cold	65.90	141.69	162.06	187.88	183.08	57.73	23.21
4 gm/kgm, room T.	69.77	187.20	240.10	297.04	414.08	487.40	283.32
4 gm/kgm, cold	92.76	176.92	188.68	238.62	257.17	302.86	113.98

Table 3-b (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data.

Source of Variance	Interval of CO_2 collection						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.	24 hrs.
A	1.05	1.05	1.30	1.45	2.77	9.74**	19.49**
B	$2.5x10^{7}$**	$1.5x10^{6}$**	$2.6x10^{3}$**	6.27**	4.03*	7.92**	16.82**
AB	1.06	1.11	1.07	1.15	1.05	1.13	1.26

A: Effect of temperature; B: Effect of treatment; AB: Interaction

*$P<0.05$

**$P<0.01$

Table 3-b (continued)

Duncan - Kramer (18, 53) test of significance to determine the effect of treatment by combining groups kept under different environmental conditions but receiving different treatment.

1 hr.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	3364	5587	8126
2 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	5880	13349	18206
3 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	3482	16617	21439
4 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	1538	19365	26783
6 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	817	23163	33562
9 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	330	10924	40800
24 hrs.	Group	0.8 gm	2.4 gm	4.0 gm
	Mean	111	3283	19865

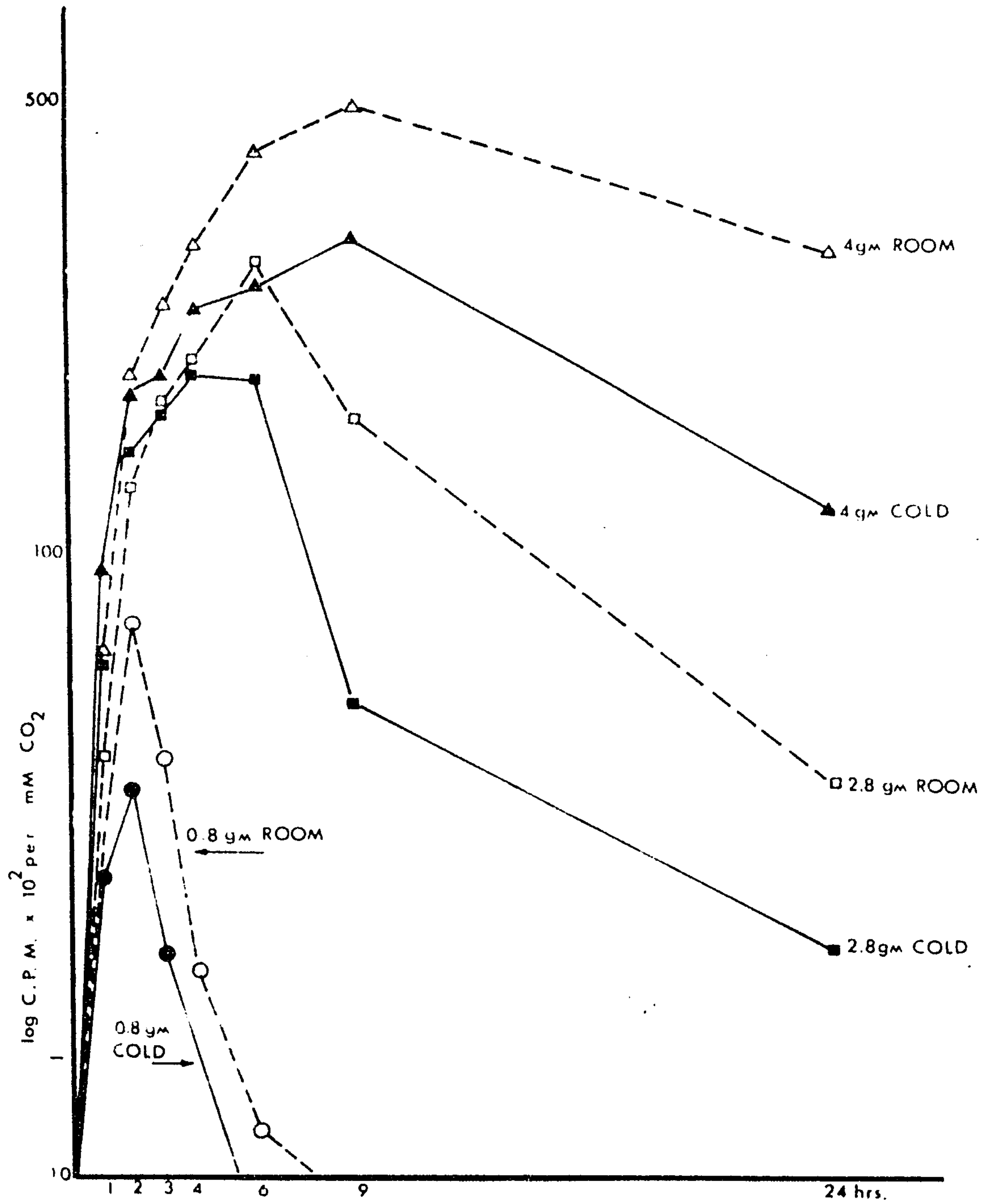

Figure 3-b. The mean respiratory CO_2 activity (c.p.m. x dilution factor per mM of CO_2) plotted as an exponential versus time in rats given variable doses of alcohol and exposed to cold or room temperature.

Table 3-c

Recovery of inert alcohol and radioactive carbon from urine collected for 24 hours and expressed as the percentage of $^{14}C_2H_5OH$ and of inert alcohol administered

Treatment	Alcohol		^{14}C	
	Room T.	Cold	Room T.	Cold
1 ml	1.42	0.64	2.27	1.04
3 ml	6.38	5.28	6.53	6.93
5 ml	7.14	5.70	6.76	6.78

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	Alcohol	^{14}C
Effect of treatment	12.189**	13.281**
Effect of temperature	1.277	0.084
Interaction	2.798	0.037

** $P < 0.01$

Table 3-c (continued)

Duncan - Kramer (18,53) test of significance of combined groups receiving identical treatment but exposed to different environmental temperatures (P = 0.01)

Alcohol in urine:

Group	0.8 gm	2.4 gm	4 gm
Mean	1.03	5.83	6.42

^{14}C in urine:

Group	0.8 gm	2.4 gm	4 gm
Mean	1.65	6.73	6.77

DISCUSSION

A survey of the data of the present experiment shows that alcohol, when given in various doses, is metabolized at similar rates for as long as six hours following its administration. Identical results were reported by Jacobsen (42) and many others. Results contrary to ours were reported by Fazekas (21). In his work, rats were given 1, 1.5 and 2 gm of alcohol per kilogram of body weight. He found that the peak rate was reached at 1, 2 and 3 hours respectively, following administration of each of these doses. The present results are also in contradiction with the findings of Vitale, _et al_ (96) and with the report made by Segovia-Riquelme, _et al_ (81).

According to Dontcheff and Porter (17), alcohol administered to rats kept at room temperature is utilized in definite proportion to the amount of foodstuff oxidized at the same time and is independent of the dose of alcohol. Thus, they infer that the stability of the ratios demonstrate a direct relationship between the velocity of alcohol utilization and the extent of the respiratory exchanges of the organism. Our results are contradictory to the latter statement. We have clearly shown a dose dependence of the ratio of oxidation of alcohol to the total foodstuff utilization at the same time. These ratios, which we refer to as the specific activity of $^{14}CO_2$ were different between the groups of animals given various doses of alcohol.

In humans, even moderate amounts of alcohol cause considerable dilatation of the skin vessels, producing flushing and a distinct sensation of warmth. This effect prevents the normal cutaneous vasoconstriction of exposure to cold, so that intoxi-

cation hastens the fatal outcome in "freezing to death". Central vasomotor depression probably plays a major role in the production of this peripheral vasodilatation, because the concentrations of alcohol found in the body fluids have little effect directly on blood vessels (21).

The centers controlling heat production and heat conservation, i.e., the mechanism whereby an animal is enabled to maintain a normal body temperature when exposed to cold, are situated in the hypothalamus. The hypothalamic center apparently exerts its controlling influence upon temperature through the transmission of sympathetic impulses to the cutaneous vessels and pilomotor muscles via the autonomic nervous system. The removal of the sympathetic nervous system renders an animal highly susceptible to cold. The depression of hypothalamic centers through high levels of alcohol might thus result not only in the abolition of responses from the exteroceptors but also in the depression of autonomic and endocrine components. In animals given high doses of alcohol and exposed to cold, the decrease in body temperature could result from continuous vasodilatation. In addition to this, there might be some central effect as well.

Additional information could be obtained by concomitant oxygen consumption rate, catecholamine determination and measurements of temperature in various parts of the body during exposure to cold and alcohol.

EXPERIMENT IV

METABOLISM OF ALCOHOL IN PARTIALLY HEPATECTOMIZED RATS EXPOSED TO COLD

In order to investigate to what extent the liver is responsible in cold-exposed animals for their increased capacity to metabolize ethyl alcohol and to determine also if a cold environment could affect the ability of the regenerating liver to metabolize alcohol, the following experiment was undertaken.

MATERIALS AND METHODS

A-Preliminary Experiment

Before the main problem could be investigated it was necessary to establish when, following partial hepatectomy, the rats could be exposed to cold with a minimum of mortality. According to Getachew-Bolodia (26) the resistance of partially hepatectomized rats exposed to a temperature of -5°C varies with the time lapse between surgical intervention and exposure to cold. Thus, subtotally hepatectomized rats, exposed 48 hours after operation, had the remarkable survival rate of 80 per cent at the end of 14 days in contrast to 50 per cent of sham-operated, and in contrast to the groups exposed to cold 3, 6, 12 and 72 hours after partial hepatectomy.

1-Materials and Methods

Rats weighing between 280 and 320 grams were divided into seven groups of eighteen animals each. The treatments

were as follows:

1st group: non-operated control;

2nd group: sham-operated and exposed to cold 12 hours after surgery;

3rd group: sham-operated and exposed to cold 48 hours after surgery;

4th group: sham-operated and exposed to cold 5 days after surgery;

5th group: partially hepatectomized and exposed to cold 12 hours after surgery;

6th group: partially hepatectomized and exposed to cold 48 hours after surgery;

7th group: partially hepatectomized and exposed to cold 5 days after surgery.

The sham-operation consisted of an incision of the abdominal wall along the linea-alba, the removal of the xiphoid process and the delivery of the median and lateral lobes of the liver followed by their return into the abdominal cavity. Partial hepatectomy was performed basically according to the method of Higgins and Anderson (39), except that the xiphoid process was also removed.

The animals were kept at -5°C for 14 days, weighed daily and their liver weight recorded at the moment of death or at the end of the experiment.

2-Results

The data given in Table 4-p (p. 66) and Figure 4-p (p. 67) compare favorably with those reported by Getachew-Bolodia (26) except for the non-operated control group where

a lower mortality rate was obtained in the current study.

B-Main Experiment

The animals used in the metabolic study of alcohol were divided into six groups of ten rats per group. The first three groups consisted of partially hepatectomized, sham-operated, and non-operated control animals which were placed in a cold room at a temperature of -5°C, 48 hours after surgical intervention where they remained for a period of five days. The other three groups, divided and operated upon as above were kept at room temperature (20° ± 2°C) for seven days.

On the eighth post-operative day each animal was injected with radioactive alcohol by intraperitoneal route. Respiratory ^{14}C carbon dioxide was collected at intervals of 1, 3, 5, 7 and 9 hours following alcohol administration. The dose of alcohol administered and the method used for the determination of its metabolic rate were previously described (p. 11).

Table 4-p

Percentage of survival of partially hepatectomized, sham-operated and non-operated control rats exposed to -5°C for 14 days at various times after surgery

Group	Survival
Non-operated control	66.7 p.c.
Sham-operated, exposed to cold 12 hours after surgery	44.4 p.c.
Sham-operated, exposed to cold 48 hours after surgery	50.0 p.c.
Sham-operated, exposed to cold 5 days after surgery	44.4 p.c.
Partially-hepatectomized, exposed to cold 12 hrs. after surgery	5.5 p.c.
Partially-hepatectomized, exposed to cold 48 hrs. after surgery	62.5 p.c.
Partially-hepatectomized, exposed to cold 5 days after surgery	31.6 p.c.

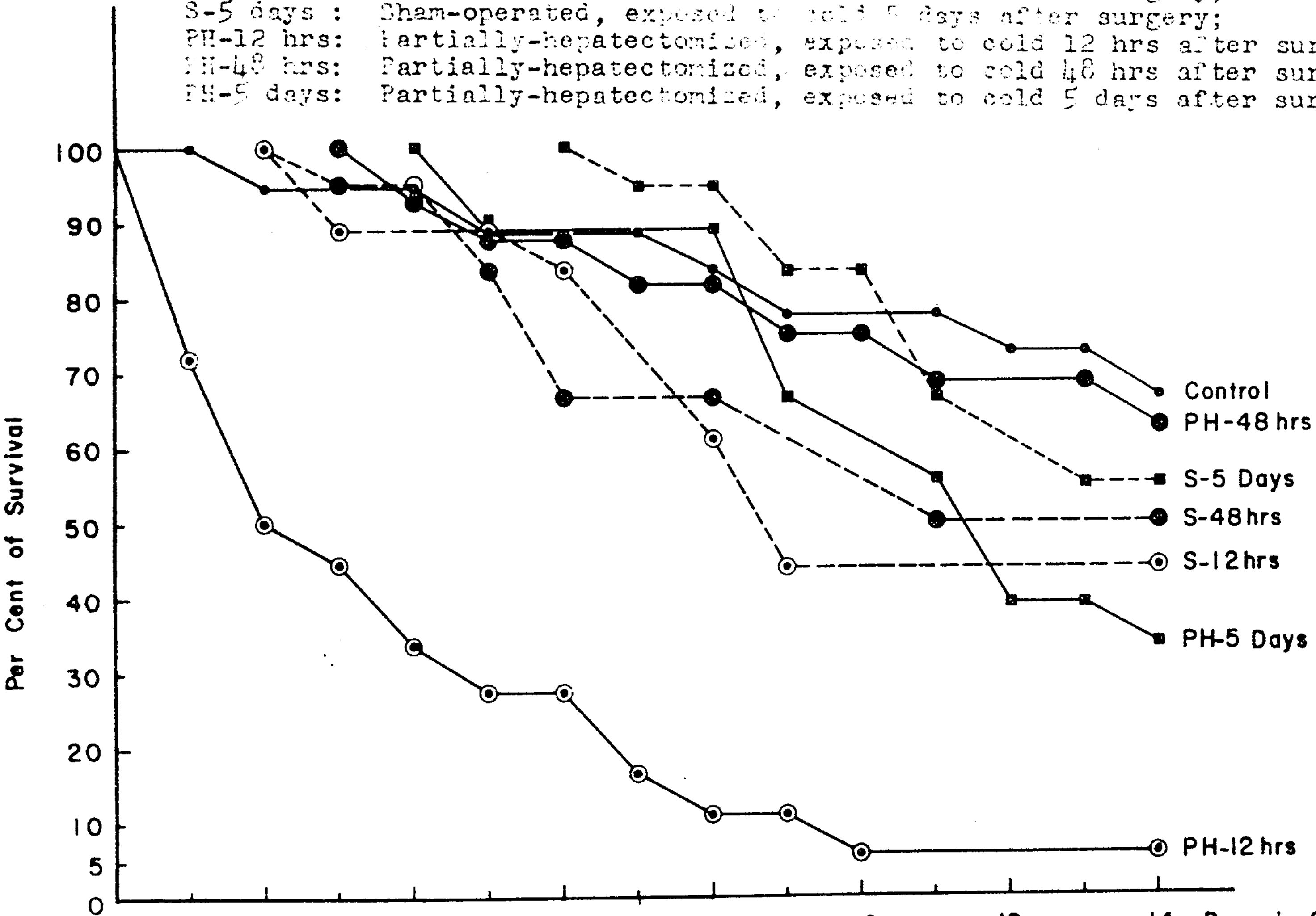

Figure 4-p. Percentages of survival of partially hepatectomized (PH), sham-operated (S) and non-operated control rats exposed -5°C for 14 days, while various time elapsed between the surgery and the beginning of the cold exposure.

RESULTS

I-Respiratory $^{14}CO_2$

A-Cumulative Recoveries

Table 4-a (p. 70) and Figure 4-a (p. 72) represent the cumulative averages of respiratory $^{14}CO_2$ production, following the administration of $^{14}C_2H_5OH$. The most striking difference in alcohol utilization rate was noted during the first three hours. It took three hours for the animals exposed to cold, regardless of the operation to which they had been subjected, to burn an amount of alcohol equivalent to that oxidized in nine hours by the rats kept at room temperature. The total nine hour collection in cold-exposed animals was significantly higher than that of their room temperature controls. There was, however, no difference at any stage of the experiment within the groups kept in the cold or at room temperature, although both cold or room temperature groups included a group of intact, sham-operated and partially hepatectomized rats.

No attempt was made to correlate the weight of the livers at autopsy in hepatectomized rats with the corresponding sham-operated or non-operated groups with regard to metabolic coefficient based on the difference of liver mass.

B-Specific Activity

Data on the specific activity of $^{14}CO_2$ are given in Table 4-b (p. 73) and Figure 4-b (p. 75). Results show a pattern similar to that observed in previous experiments. There was no difference between the cold- and warm-exposed rats after the first hour, but significant differences occurred be-

tween them at subsequent periods. Thus, the rats kept at room temperature oxidized more alcohol in proportion to their general metabolism than their counterparts exposed to cold. No difference in this set of analysis was observed between different treatments at either temperature of exposure.

II-Body Weight

The changes in body weight are given in Table 4-c (p. 76).

Rats exposed to cold continously lost weight, whereas the normal temperature groups all gained weight. No statistically significant differences were found between treatment groups at either exposure temperature.

III-Liver Weight

Table 4-d (p. 77) are the values of liver weight at autopsy, immediately after the metabolic study of alcohol. Although the liver weight of partially hepatectomized rats exposed to cold appeared to be much lower than in the non-treated controls or sham-operated group, the differences were not statistically significant.

Table 4-a

Mean values of cumulative recoveries of mM of CO_2 from metabolized alcohol.

Treatment	Interval of CO_2 collection				
	1 hr.	3 hrs.	5 hrs.	7 hrs.	9 hrs.
Control, room T.	0.71	3.60	4.42	4.70	4.86
Control, cold	1.30	4.83	5.32	5.49	5.57
Sham, room T.	0.78	3.52	4.50	4.80	4.95
Sham, cold	1.36	4.84	5.44	5.64	5.73
Part. hep., room T.	0.63	3.23	4.30	4.58	4.73
Part. hep., cold	1.33	4.70	5.16	5.39	5.47

Table 4-a (contined)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of Variance	Interval of CO_2 collection				
	1 hr.	3 hrs.	5 hrs.	7 hrs.	9 hrs.
A	0.264	0.532	0.554	0.858	1.024
B	31.979**	39.372**	35.094**	31.089**	28.476**
AB	0.118	0.113	0.007	0.010	0.011

A: Effect of treatment; B: Effect of temperature; AB: Interaction; ** $P < 0.01$

Averages and Student's t values of combined groups kept at room temperature and of groups exposed to cold, without regard to treatment

	Interval of CO_2 collection				
	1 hr.	3 hrs.	5 hrs.	7 hrs.	9 hrs.
Room T.	0.71	3.46	4.41	4.70	4.86
Cold	1.33	4.79	5.33	5.50	5.59
Student's t	9.682**	13.945**	10.883**	9.072**	8.143**

** $P < 0.01$

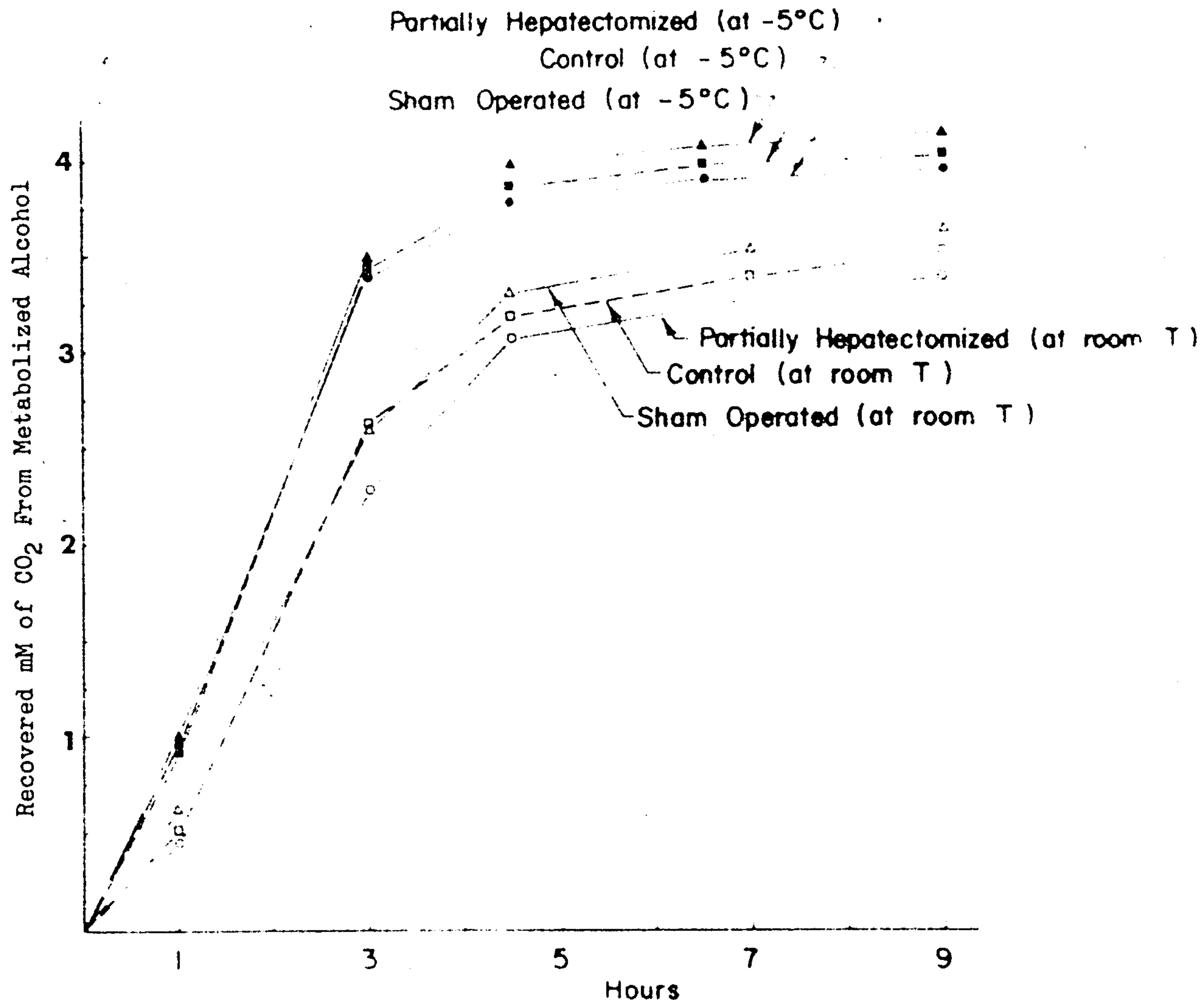

Figure 4 - a. Cumulative recoveries of mM of CO_2 from metabolized alcohol, administered to rats exposed to cold and to others kept at room temperature.

Table 4-b

The specific activity of $^{14}CO_2$, represented as number of counts per minute per millimol of expired CO_2.

Treatment	Interval of CO_2 collection				
	1 hr.	3 hrs.	5 hrs.	7 hrs.	9 hrs.
Control, room T.	3829	7851	2291	762	424
Control, cold	3839	4980	770	241	130
Sham, room T.	3807	8420	2923	973	521
Sham, cold	4066	5339	837	296	148
Part. hep., room T.	2907	7255	2708	813	423
Part. hep., cold	3817	4916	708	221	118

Table 4-b (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of Variance	Interval of CO_2 collection				
	1 hr.	3 hrs.	5 hrs.	7 hrs.	9 hrs.
A	2.771	0.879	0.341	0.626	0.474
B	3.585	32.853**	28.906**	31.337**	35.134**
AB	1.645	0.203	0.264	0.180	0.186

A: Effect of treatment; B: Effect of temperature;
AB: Interaction; ** $P < 0.01$

Averages and Student's t values of combined groups kept at room temperature and of groups exposed to cold, without regard to treatment

	Interval of CO_2 collection				
	1 hr.	3 hrs.	5 hrs.	7 hrs.	9 hrs.
Room T.	3558	7861	2639	851	460
Cold	3900	5064	770	250	132
Student's t	1.770	10.826**	8.932**	9.615**	11.370**

** $P < 0.01$

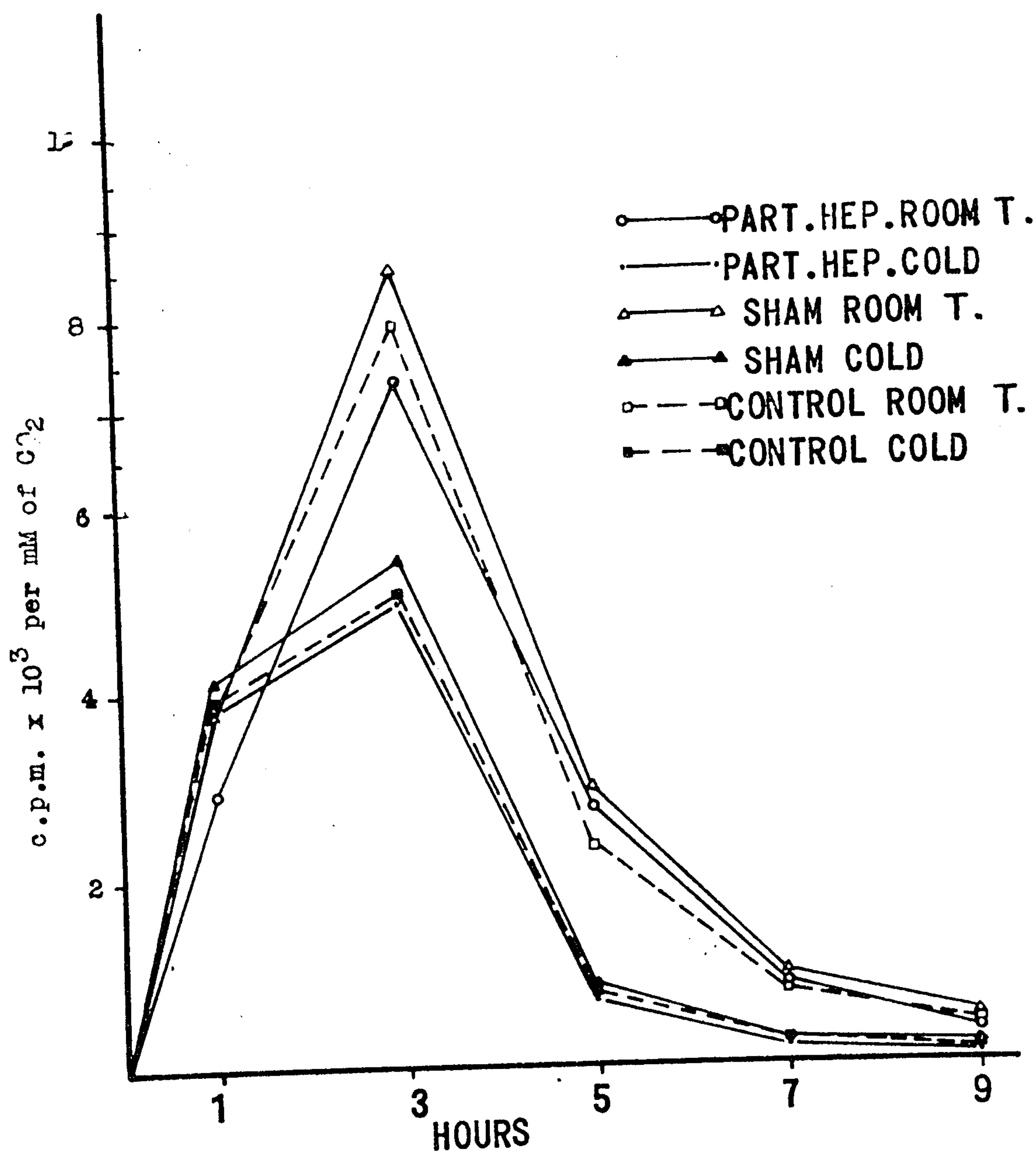

Figure 4 - b. The specific activity of $^{14}CO_2$ at different intervals following injection of radioactive alcohol to rats exposed to cold and to others kept at room temperature. Results are expresse.. is the number of counts per minute per millimol of expired CO_2.

Table 4-c

Mean values of the differences in body weight (in grams) between the first and the last day of cold exposure or an equivalent time at room temperature

Treatment	Kept at room temperature	Cold-exposed
Non-operated control	27.50	-22.50
Sham-operated	24.60	-24.33
Partial hepatectomy	20.33	-12.22

F values calculated from Yates' (109) Analysis of Variance for the above data

Effect of treatment	0.104
Effect of temperature	38.233^{**}
Interaction	0.729

** $P < 0.01$

Mean values and Student's t of groups kept at room temperature and of groups exposed to cold, without regard to treatment

Room T	24.27
Cold	-20.86
Student t	13.182^{**}

** $P < 0.01$

Table 4-d

Mean values of liver weight (in grams) at autopsy

Treatment	Kept at room temperature	Cold-exposed
Non-operated control	9.92	9.34
Sham-operated	8.58	9.31
Partial hepatectomy	7.85	6.70

F values calculated from Yates' (109) Analysis of Variance for the above data

Effect of treatment	9.178**
Effect of temperature	0.368
Interaction	1.499

** $P < 0.01$

Duncan - Kramer (18, 53) test of significance for the effect of treatment

Group	Part. hep.	Sham	Control
Mean	7.28	8.93	9.53

Table 4-d'

Removed liver weight (in grams) at surgery

Animals kept at room temperature	6.29 ± 0.20 gm
Animals exposed to cold	6.12 ± 0.08 gm
Student's t	0.481

DISCUSSION

In recent years considerable work has shown that the increased capacity of the cold-exposed rat to produce heat is reflected in an elevated in vitro oxygen consumption of certain tissues such as the liver (14, 34, 100). The temperature of the liver is higher than that of any other organ in the body (97). It has been suggested by Weiss (100) that when a homeotherm is exposed to sufficient cold to produce adaptive changes, the various tissues of the body do not acclimatize at the same rate; indeed, the liver seems to be the first tissue to increase its rate of oxygen consumption, although skeletal muscles also increase their oxygen consumption through shivering. On the other hand the bulk of evidence as to the preferential site of alcohol metabolism indicates that it is the liver (43).

It has been demonstrated in the present experiment that exposure of rats to rather acute cold results in an elevated metabolism of ethyl alcohol. This was particularly pronounced during the first three hours, i.e. the period when the greatest portion of alcohol was metabolized; but the relative metabolism of alcohol, as measured by the specific activity of $^{14}CO_2$, was depressed in all groups of animals exposed to cold. Weiss and Moss (102) reported that cold environment does not interfere with the rat's ability to regenerate the liver mass. Similar observations were made in the present experiment. The latter workers also reported that the oxidative activity of the liver was increased in partially hepatectomized rats, and still more so if they were cold-exposed.

They were, however, unable to find any significant difference between the oxidative metabolism of liver slices taken from cold-exposed-hepatectomized rats and cold-exposed controls.

It was of considerable interest to find in the foregoing experiment that the weight of the liver of partially hepatectomized rats was obviously smaller at autopsy, than that of the controls. Nevertheless, the rate of metabolism of alcohol in those animals was similar to that observed in sham-operated and controls. This was true for either cold-exposed or room temperature maintained animals.

The increased metabolism of alcohol by the liver of cold-exposed rats might be a measure of the increased over-all metabolic rate of this organ; it metabolized all substrates more rapidly, including alcohol. However, when the contribution of alcohol to the general metabolism of the whole animal is determined (specific activity), it is decreased as compared to room temperature controls. This would confirm the conclusion of other workers, to the effect that tissues other than the liver contribute little to the metabolism of alcohol.

EXPERIMENT V

THE EFFECT OF THE ADRENERGIC BLOCKING AGENT, PHENOXYBENZAMINE, ON THE METABOLISM OF ALCOHOL IN RATS EXPOSED TO COLD

The purpose of the following experiment was to establish to what extent the catecholamines were involved in increased metabolism of ethyl alcohol as observed in cold-exposed rats.

MATERIALS AND METHODS

Rats were divided into ten groups of at least six animals each. The first five groups were exposed to 2°C for 5 days prior to metabolic studies of alcohol and were treated as follows:

Group 1: non-treated control;

Group 2: received propylene glycol (a diluent of phenoxybenzamine•HCl) once only prior to alcohol administration;

Group 3: received propylene glycol daily;

Group 4: received phenoxybenzamine•HCl* once only prior to alcohol administration;

Group 5: received phenoxybenzamine•HCl daily.

Groups 6-10 were distributed and treated as those above but were kept at room temperature for the duration of the experiment.

*Phenoxybenzamine•HCl was generously supplied under the trade marks of Dibenzyline•HCl by Smith, Kline and French Interamerican Corporation, Montreal, Que.

On the sixth day each animal was administered radioactive alcohol. The dose of alcohol administered and the methods of determination of its metabolic rate were as previously described (p. 11). Respiratory ^{14}C carbon dioxide and urine were collected for nine hours following alcohol administration.

Phenoxybenzamine HCl (prepared by dissolving 10 mgm of phenoxybenzamine HCl per ml of propylene glycol) was administered at a dose of 5 mgm per kgm of body weight.

RESULTS

I-Respiratory $^{14}CO_2$

A-Cumulative Recoveries

As in previous experiments, the rate of alcohol metabolism in animals exposed to cold and not submitted to any treatment was different from their controls kept at room temperature (Table 5-a, Figure 5-a). Combined cold exposure and treatment with phenoxybenzamine, however, gave different results. When the adrenergic blocking agent was given only once prior to alcohol administration, the combustion rate of alcohol was lower than in untreated cold-exposed animals but slightly higher than those kept at room temperature; in rats pretreated daily for one week with phenoxybenzamine, the rate of alcohol metabolism was further depressed as compared to their cold-exposed untreated controls and was similar to that of their room temperature controls.

The difference in alcohol metabolism was particularly significant between groups of pretreated animals kept under two different environmental conditions. No differences in the rate of alcohol metabolism were observed, however, within the groups kept at room temperature.

B-Specific Activity

In Table 5-b (p. 90) and Figure 5-b (p. 94) the specific activity of respiratory $^{14}CO_2$ at each collection period after alcohol administration is given for each group of rats. It is clear from the data that in cold-exposed animals, whatever the treatment, the ratio of the total respiratory CO_2 to $^{14}CO_2$ derived from alcohol was significantly lower

throughout the duration of the experiment than their room temperature controls. No discernible difference was noted within the groups of cold-exposed rats. In animals kept at room temperature and given phenoxybezamine once prior to alcohol, however, a lower specific activity of $^{14}CO_2$ was observed. This was especially noticeable during the first three hours. This tendency was further accentuated in pre-treated animals.

II-Urinary Excretion of Alcohol

The recoveries of alcohol and ^{14}C in urine are given in Table 5-c (p. 95).

Animals exposed to cold excreted significantly less alcohol in their urine than did their controls at room temperature. Phenoxybenzamine treated rats eliminated significantly more alcohol than did their non-treated counterparts. The same is true for the room temperature group as well as for cold-exposed rats.

Similar results to the above were observed for the recovery of ^{14}C in urine.

Table 5-a

Cumulative mean recoveries of CO_2 (in mM) from metabolized alcohol, administered to rats exposed to cold and to others kept at room temperature.

Treatment	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
Control, room T.	0.80	2.63	3.71	4.18	4.59	4.87
Control, cold	1.38	3.99	4.95	5.26	5.61	5.88
Solvent once, room T.	0.84	2.62	3.70	4.46	4.93	5.33
Solvent once, cold	1.40	3.55	4.47	4.88	5.27	5.48
Solvent daily, room T.	0.81	2.35	3.65	4.46	5.11	5.53
Solvent daily, cold	1.60	4.01	4.88	5.29	5.65	5.88
Drug once, room T.	0.74	2.22	3.37	4.08	4.58	5.00
Drug once, cold	1.14	3.31	4.29	4.74	5.11	5.32
Drug daily, room T.	0.63	2.12	3.32	3.96	4.48	4.88
Drug daily, cold	0.98	2.58	3.85	4.54	5.11	5.42

Table 5-a (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of Variance	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
A	2.293	3.122*	2.808*	2.417	2.183	2.044
B	31.701**	29.521**	29.709**	23.704**	18.508**	11.539**
AB	0.657	1.014	0.659	0.569	0.614	1.101

A: Effect of treatment; B: Effect of temperature; AB: Interaction

* $P<0.05$

** $P<0.01$

Table 5-a (continued)

Duncan - Kramer (18, 53) test of significance of combined groups receiving identical treatment but exposed to different environmental temperatures. Only those intervals of CO_2 collections are indicated which have an F value of $P<0.05$

		Drug daily	Drug once	Solvent once	Solvent daily	Control
2 hours	Mean	2.35	2.77	3.16	3.18	3.45

		Drug daily	Drug once	Solvent once	Solvent daily	Control
3 hours	Mean	3.59	3.83	4.08	4.26	4.46

Table 5-a (continued)

Averages and Student's t values of combined groups kept at room temperature and of groups exposed to cold, without regard to treatment.

	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
Room T.	0.76	2.36	3.53	4.20	4.71	5.10
Cold	1.28	3.47	4.48	4.94	5.34	5.60
Student's t	8.603**	8.445**	8.628**	6.985**	5.801**	4.272**

** $P<0.01$

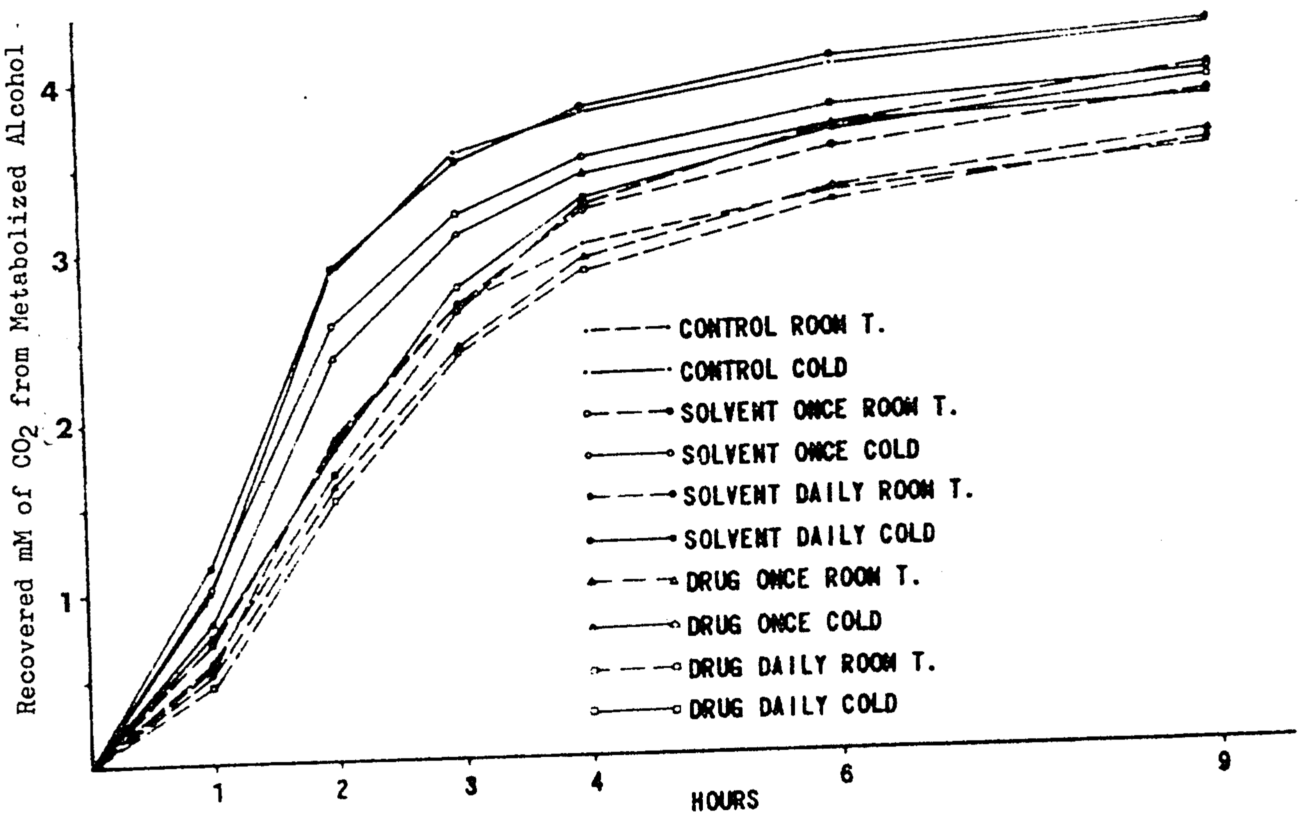

Figure 5 - a. Cumulative recoveries of CO_2 (in mM) from metabolized alcohol, administered to rats exposed to cold and to others kept at room temperature.

Table 5-b

Mean values of $^{14}CO_2$ specific activity, represented as c.p.m. per mM of exhaled carbon dioxide.

Treatment	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
Control, room T.	3641	7571	4674	2149	1194	505
Control, cold	3087	4190	2270	928	440	156
Solvent once, room T.	3243	7010	5321	2722	1437	624
Solvent once, cold	2666	4151	2623	1190	580	231
Solvent daily, room T.	3862	6614	5208	2250	1658	643
Solvent daily, cold	3018	4048	2576	1135	608	213
Drug once, room T.	2593	5846	4593	2830	1293	622
Drug once, cold	2418	4174	1940	793	347	158
Drug daily, room T.	2297	5274	4411	2191	1156	519
Drug daily, cold	2558	4064	3438	1737	833	324

Table 5-b (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data.

Source of variance	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
A	6.775**	1.575	0.622	0.502	0.825	0.415
B	5.268*	44.356**	38.654**	30.351**	35.341**	39.702**
AB	1.411	1.371	0.888	1.439	0.978	0.738

A: Effect of treatment; B: Effect of temperature; AB: Interaction

* $P<0.05$

** $P<0.01$

Table 5-b (continued)

Duncan - Kramer (18, 53) test of significance of combined groups receiving identical treatment but exposed to different environmental temperatures. Only that interval of CO_2 collection is indicated which has an F value of $P<0.05$.

1 hour

Group	Drug daily	Drug once	Solvent once	Control	Solvent daily
Mean	2426	2505	2955	3310	3440

Table 5-b (continued)

Averages and Student's t values of combined groups kept at room temperature and of groups exposed to cold, without regard to treatment

	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
Room T.	3047	6357	4792	2437	1334	582
Cold	2749	4131	2562	1151	555	213
Student's t	1.980	11.668**	10.779**	8.506**	9.649**	11.608**

** $P<0.01$

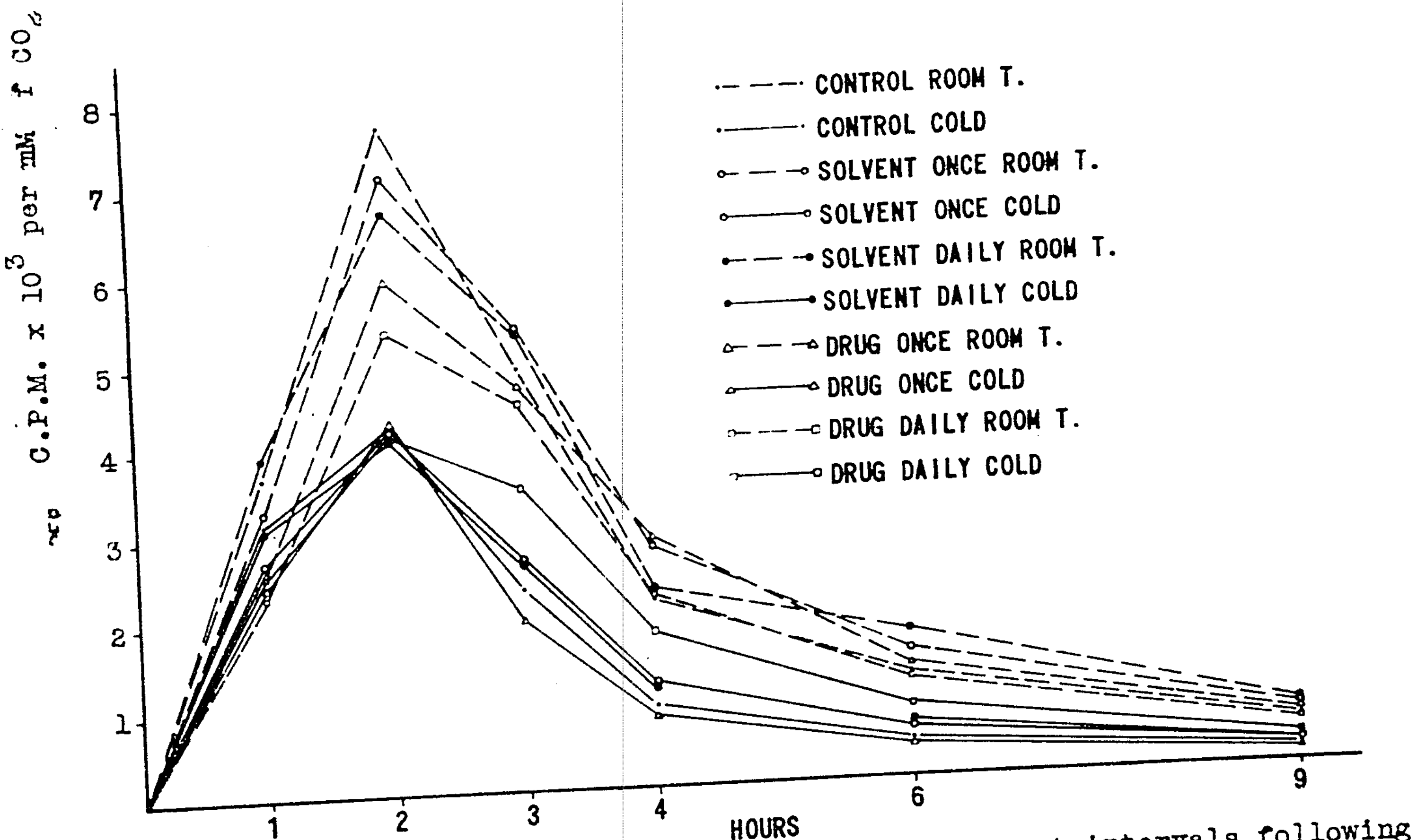

Figure 5 - b. The specific activity of $^{14}CO_2$ at different intervals following injection of radioactive alcohol to rats exposed to cold and to others kept at room temperature. Results are expressed as the number of counts per minute per m limol of expired CO_2.

Table 5-c

Mean values of the recovery of ^{14}C and of inert alcohol in urine collected for 9 hours and expressed as the percentage of $^{14}C_2H_5OH$ and of inert alcohol administered

Treatment	^{14}C in urine		Alcohol in urine	
	Room T.	Cold	Room T.	Cold
Control	2.27	1.09	1.42	0.77
Solvent once	1.93	1.44	1.55	0.94
Solvent daily	2.17	1.10	1.59	0.77
Drug once	3.07	2.66	2.43	1.76
Drug daily	3.72	2.82	3.03	1.62

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	^{14}C in urine	Alcohol in urine
Effect of treatment	7.070**	5.749**
Effect of temperature	9.552**	15.052**
Interaction	0.346	0.477

** $P<0.01$

Table 5-c (continued)

Duncan - Kramer (18, 53) test of significance of combined groups receiving identical treatment but exposed to different environmental temperatures

^{14}C in urine	Group	Solvent daily	Solvent once	Control	Drug once	Drug daily
	Mean	1.64	1.68	1.68	2.86	3.27

Alcohol in urine	Group	Control	Solvent daily	Solvent once	Drug once	Drug daily
	Mean	1.09	1.18	1.24	2.09	2.32

($P = 0.05$)

Mean and Student's t values of ^{14}C recovery and of alcohol in urine of groups of animals kept at room temperature and of groups exposed to cold, without regard to treatments

	^{14}C in urine	Alcohol in urine
Kept at room T.	2.63	2.00
Exposed to cold	1.82	1.17
Student's t	3.701**	4.998**

** $P < 0.01$

DISCUSSION

The importance of the role of catecholamines in increasing the rate of metabolism of alcohol in rats exposed to cold was demonstrated in the present experiment with phenoxybenzamine. It was found that a single dose of the adrenergic blocking agent, prior to alcohol administration, decreased the alcohol metabolism; whereas, daily administration of phenoxybenzamine produced a sharp reduction in alcohol metabolism. This decrease in the alcohol combustion rate was pronounced at the second hour of CO_2 collection in cold-exposed animals when they were subjected to one week of daily treatment of phenoxybenzamine.

It is difficult to assert which one of the catecholamines is responsible for this alteration in alcohol metabolism, since phenoxybenzamine is specific neither for adrenaline nor for noradrenaline.

It is not surprising to see a decreased alcohol metabolism in rats exposed to cold and treated either once or daily with phenoxybenzamine during this cold exposure. Phenoxybenzamine by virtue of its non-competitive blockade of the adrenergic mediator prevents access of the mediator to the receptor and thus may inhibit an increased mobilization of energy substrates normally occuring upon exposure of homeotherms to cold. As a consequence of this depression, the normally enhanced metabolic rate may be lowered. Therefore, we may assume that the increased metabolism of alcohol in cold is not directly related to the action of catecholamines, but may be associated with an increased general metabolism, in which the catecholamines play important roles.

EXPERIMENT VI

THE EFFECT OF POTASSIUM PERCHLORATE ON THE METABOLISM OF ALCOHOL IN COLD-EXPOSED RATS

The purpose of the present experiment was to determine to what extent, if any, the thyroid gland contributed to the increased metabolism of ethyl alcohol in cold-exposed rats.

MATERIALS AND METHODS

Rats weighing 190 to 210 gm were randomly divided into six groups of six.

Group 1 was acclimatized at 2°C for 30 days and then given a saturated aqueous solution of potassium perchlorate as a drinking solution for five days;

Group 2 was treated as Group 1, but did not receive any perchlorate;

Group 3 was reared for one month at room temperature and then transferred to a cold room at 2°C for five days. During these five days they received a potassium perchlorate solution for drinking;

Group 4 was treated as Group 3, but did not receive a potassium perchlorate solution;

Group 5 was reared throughout the experiment at room temperature and given potassium perchlorate during the last five days;

Group 6 was a normal control group reared at room temperature and received no treatment of any kind.

At the conclusion of the treatment a metabolic study of alcohol was made. The dose of alcohol and the procedures utilized in the measurement of its metabolism were as previously described (p. 11) with the difference that perchlorate was administered during the period of alcohol metabolism measurements. $^{14}CO_2$ and urine were collected for 9 hours.

At the conclusion of the experiment the body weight gain of each animal was recorded and the rats sacrificed. The thyroid glands were removed and weighed and histological sections prepared and studied according to the method of DesMarais and LaHam (16).

RESULTS

I-Respiratory $^{14}CO_2$

A-Cumulative Recoveries

In Table 6-a (p. 103) and Figure 6-a (p. 107) the data are compiled as the cumulative averages of $^{14}CO_2$ in mM, following administration of $^{14}C_2H_5OH$. As in the previous experiments in untreated rats exposed to cold for a short period of time or in cold-acclimated ones, the metabolic rate of alcohol was accelerated in contrast to controls kept at room temperature. When perchlorate-treated animals were taken into consideration, the picture became more complex. F values for the effect of environmental temperature were significant at least at the 5 per cent level at every stage of ^{14}C collection. When the latter data were submitted to the Duncan - Kramer (18, 53) test for temperature effect only, irrespective of $KClO_4$ treatment, except for the first and ninth hours of ^{14}C measurement, the only difference noted occurred between the groups exposed to cold for five days and those rats kept at room temperature. By grouping the rats into perchlorate-treated and non-treated groups, irrespective of environmental conditions, the data clearly indicated a decreased rate of alcohol metabolism in rats pretreated with perchlorate at 1, 2 and 3 hours, following which both treated and non-treated groups had identical metabolic patterns of alcohol metabolism.

B-Specific Activity

The average specific activities of $^{14}CO_2$ from radioactive alcohol are given in Table 6-b (p. 108) and Figure 6-b (p. 111). In this relative measurement of alcohol metabolism

the results indicated a partial dependence of the combustion rate of alcohol upon the environmental conditions inasmuch as these affect the increased general metabolism and the metabolic rate of the liver in particular. Rats exposed to cold for five days and those acclimated to cold had a lower ratio or $^{14}CO_2$ to the total amount of carbon dioxide than those animals kept at room temperature. The only exception was during the first hour. Similar observations were made in Experiment I. No difference in specific activity was observed at any time between the animals with blocked thyroids and the untreated ones.

II-Urinary Excretion of Alcohol

Perchlorate treated animals eliminated via the urine significantly more ^{14}C and more alcohol than the non-treated rats. The results are given in Table 6-c (p. 112).

III-Weight of Thyroids

There was a slight increase in the weight of the thyroid glands in rats exposed to cold for five days, while those acclimated to cold had a substantial increase. Perchlorate treatment produced a significant increase in the weight of the thyroids in all groups in contrast to their non-treated controls (Table 6-d, p. 114).

IV-Histological Examination of Thyroids

Histological sections of the thyroids of the cold-acclimated, perchlorate-treated animals (Group 1) lack the typical follicular arrangement. There was hypertrophy of the glands and increased vascularization, negligible colloid and increased epithelial cell height, all of which indicated that a complete physiological block had occurred. In rats exposed to cold for five

days and given perchlorate (Group 3), the glandular hypertrophy was less pronounced than in the above group but follicular colloid was lacking. The histological picture of hypertrophy was still less pronounced in animals kept at room temperature and given perchlorate for five days; in this case hypofunction was still evident but colloid was present in minute amounts. A typical picture of the hypofunctional gland is given in Figure 6-c (p. 118).

V-Body Weight

Animals exposed to cold for a short period gained less weight than the cold-acclimated ones, and the latter showed less gain than the controls kept at room temperature. Perchlorate administration resulted in a substantial loss of weight of the treated animals in contrast to their respective controls. These results are illustrated in Table 6-e (p. 116).

Table 6-a

Mean values of cumulative recoveries of exhaled CO_2 from metabolized alcohol (mM of $^{14}CO_2$).

Treatment	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
Room T.	0.81	2.63	3.71	4.18	4.59	4.87
Room T. and $KClO_4$	0.70	2.07	3.27	4.03	4.57	4.92
Cold acclim.	1.24	3.44	4.33	4.64	4.99	5.22
Cold acclim. and $KClO_4$	0.98	3.08	3.96	4.30	4.61	4.83
Cold (5 days)	1.38	3.99	4.95	5.26	5.61	5.88
Cold (5 days) and $KClO_4$	0.97	3.21	4.37	4.88	5.17	5.35

Table 6-a (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
A	4.720*	8.367**	9.642**	9.645**	7.582**	6.212**
B	4.881*	5.086*	4.715*	2.579	2.465	2.667
AB	0.589	0.251	0.097	0.152	0.514	0.913

A: Effect of temperature; B: Effect of treatment; AB: Interaction

* $P<0.05$

** $P<0.01$

Table 6-a (continued)

Duncan - Kramer (18, 53) test of significance of combined groups receiving various treatments but kept at the same environmental temperature

1 hour	Group	Room T.	Cold acclim.	Cold (5 days)
	Mean	0.75	1.14	1.22
2 hours	Group	Room T.	Cold acclim.	Cold (5 days)
	Mean	2.35	3.39	3.68
3 hours	Group	Room T.	Cold acclim.	Cold (5 days)
	Mean	3.51	4.18	4.72
4 hours	Group	Room T.	Cold acclim.	Cold (5 days)
	Mean	4.11	4.51	5.11
6 hours	Group	Room T.	Cold acclim.	Cold (5 days)
	Mean	4.58	4.84	5.44
9 hours	Group	Room T.	Cold acclim.	Cold (5 days)
	Mean	4.89	5.06	5.67

When underlined with the full line, $P<0.01$

When underlined with the broken line, $P<0.05$

Table 6-a (continued)

Means and Student's t values of groups receiving $KClO_4$ in drinking water and of groups receiving plain tap-water, without regard to environmental temperature. Only those intervals of CO_2 collections are indicated which have a significance of $P<0.05$.

Treatment	Interval of CO_2 collection		
	1 hour	2 hours	3 hours
No $KClO_4$ added	7.18	[illegible].[illegible]	4.[illegible]
$KClO_4$ added	6.[illegible]8	[illegible].79	[illegible].87
Student's t	3.241**	3.181**	2.981**

** $P<0.01$

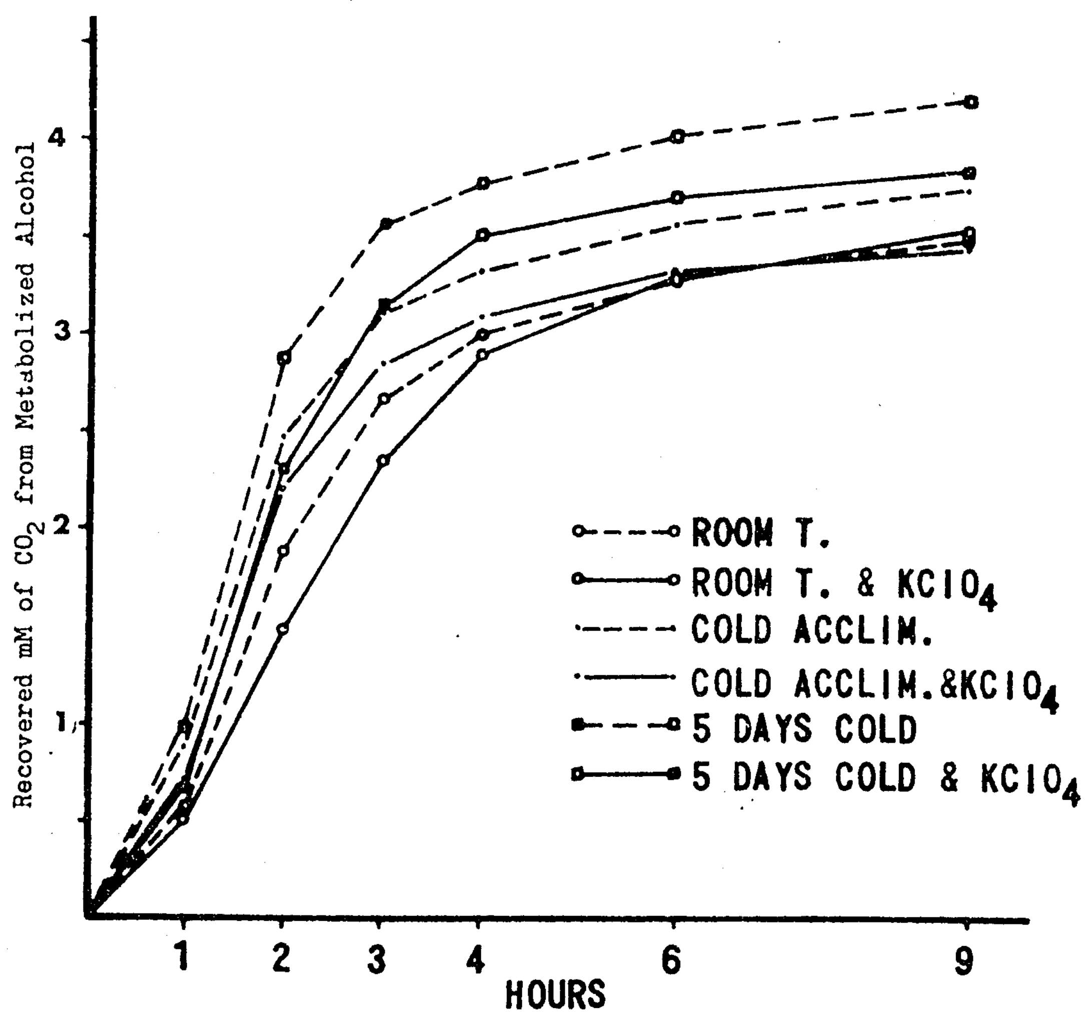

Figure 6 - a. Cumulative recoveries of CO_2 from metabolized alcohol, administered to rats exposed to cold and to others kept at room temperature.

Table 6-b

Mean values of $^{14}CO_2$ specific activity, expressed as c.p.m. per mM of exhaled carbon dioxide.

Treatment	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
Room T.	3641	7571	4674	2149	1194	505
Room T. and $KClO_4$	3215	6776	5463	3008	1303	616
Cold acclim.	3043	4680	1822	734	391	225
Cold acclim. and $KClO_4$	3148	4636	2416	914	499	219
Cold (5 days)	3087	4190	2270	928	440	156
Cold (5 days) and $KClO_4$	3083	4326	2689	1245	495	191

Table 6-b (continued)

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	Interval of CO_2 collection					
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	9 hrs.
A	0.922	13.802**	11.658**	11.223**	13.481**	13.402**
B	0.228	0.241	1.295	2.039	0.419	0.512
AB	0.471	0.339	0.039	0.404	0.018	0.261

A: Effect of temperature; B: Effect of treatment; AB: Interaction

** $P<0.01$

Table 6-b (continued)

Duncan - Kramer (18, 53) test of significance of combined groups receiving various treatments but kept at the same environmental temperature. Only those intervals of CO_2 collections are indicated which have an F value with $P<0.05$.

	Group	Cold (5 days)	Cold acclim.	Room T.
2 hours	Mean	4245	4662	7172

	Group	Cold acclim.	Cold (5 days)	Room T.
3 hours	Mean	2058	2437	5068
4 hours	Mean	805	1054	2578
6 hours	Mean	434	462	1249
9 hours	Mean	170	227	560

When underlined with the solid line, $P<0.01$

When underlined with the broken line, $P<0.05$

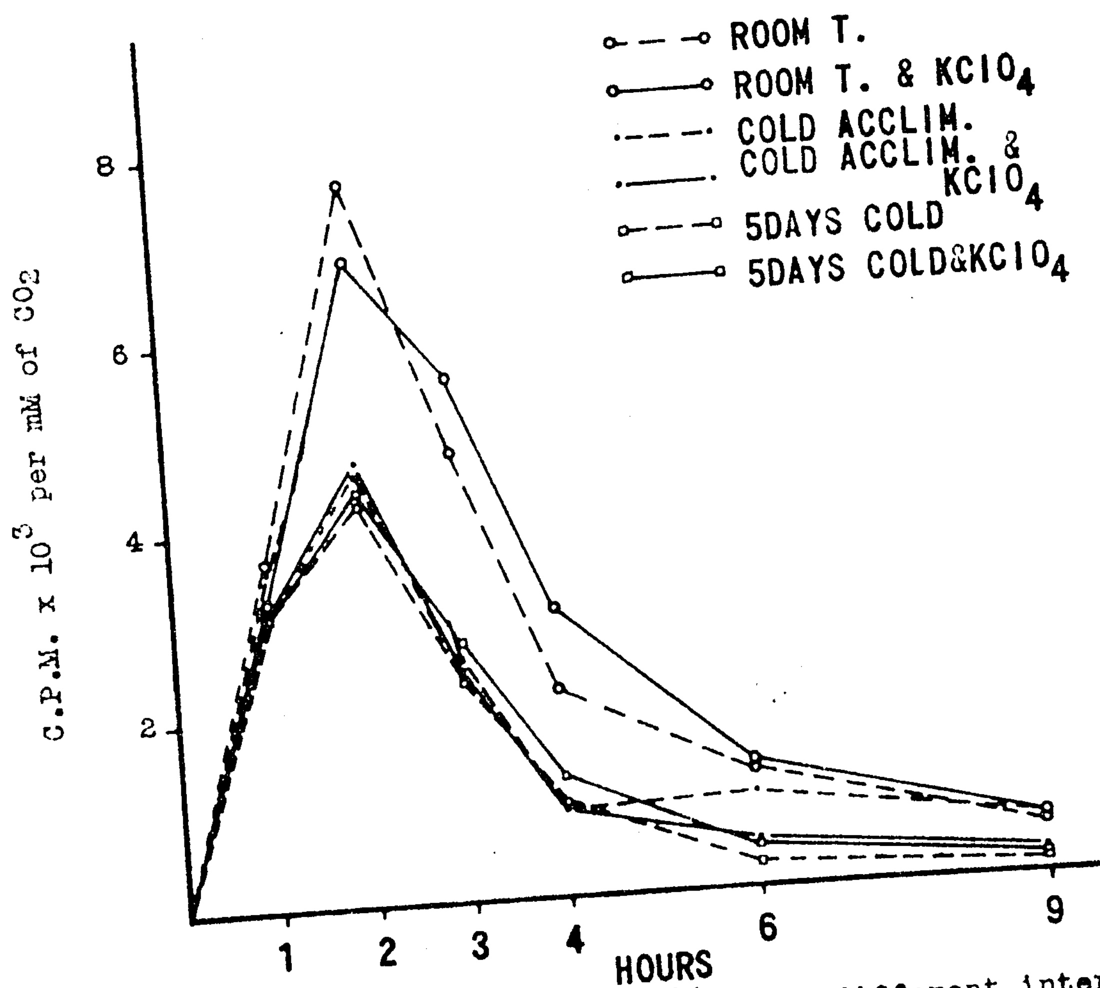

Figure 6-b. The specific activity of $^{14}CO_2$ at different intervals following injection of radioactive alcohol to rats exposed to cold and to others kept at room temperature. Results are expressed as the number of counts per minute per millimol of expired CO_2.

Table 6-c

Mean values of the recovery of ^{14}C and of inert alcohol from urine collected for 9 hours and expressed as the percentage of $^{14}C_2H_5OH$ and of inert alcohol administered

Treatment	^{14}C in urine		Alcohol in urine	
	No $KClO_4$	$KClO_4$ added	No $KClO_4$	$KClO_4$ added
Room T.	2.59	4.02	1.48	2.18
Cold acclim.	0.76	3.69	0.59	2.57
Cold (5 days)	1.04	2.99	0.64	1.73

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	^{14}C in urine	Alcohol in urine
Effect of temperature	1.848	0.990
Effect of treatment	12.638**	11.026**
Interaction	0.557	1.014

** $P<0.01$

Table 6-c (continued)

Mean values and Student's t of groups that received $KClO_4$ in their drinking water and of groups drinking tap-water only, without regard to various environmental temperatures.

	^{14}C in urine	Alcohol in urine
No $KClO_4$	1.46	0.90
$KClO_4$ added	3.57	2.16
Student's t	4.975**	4.445**

** $P<0.01$

Table 6-d

Mean values of the weight of the thyroid glands (in mgm) removed at autopsy

Treatment	No $KClO_4$	$KClO_4$ added
Room T.	13.57	24.98
Cold acclimated	23.67	39.80
Cold (5 days)	15.90	26.78

F values calculated from Yates' (109) Analysis of Variance for the above data

Source of variance	F value
Effect of temperature	5.276*
Effect of treatment	14.569**
Interaction	0.247

* $P<0.05$

** $P<0.01$

Table 6-d (continued)

Duncan - Kramer (18, 53) test of significance to determine the effect of environmental temperature by combining groups receiving various treatment but kept under the same environmental conditions

Group	Room T.	Cold (5 days)	Cold acclimated
Mean	19.27	21.34	31.74

Mean values and Student's t of groups receiving $KClO_4$ in their drinking water and of groups drinking tap-water only, without regard to various environmental conditions

No $KClO_4$	17.71
$KClO_4$ added	30.52
Student's t	5.666**

** $P<0.01$

Table 6-e

Mean values of the body weight difference (in grams) between the first and the last day of $KClO_4$ administration and of an equivalent time interval in the respective control group

Treatment	No $KClO_4$	$KClO_4$ added
Room T.	29.83	-13.50
Cold acclimated	14.83	10.00
Cold (5 days)	5.33	- 2.33

F values of Yates' (109) Analysis of Variance for the above data

Source of variance	F value
Effect of temperature	2.974
Effect of treatment	11.388**
Interaction	2.183

** $P<0.01$

Table 6-e (continued)

Mean values and Student's t of groups receiving $KClO_4$ and of groups drinking tap-water only

No $KClO_4$	16.67
$KClO_4$ added	- 8.94
Student's t	4.558**

** $P<0.01$

Figure 6-c. Histological section of a typical hypofunctional thyroid gland. (From a group of rats exposed to cold for a short period of time and pretreated with $KClO_4$)

DISCUSSION

From the results obtained in the present experiment it seems reasonably certain that the increased function of the thyroid gland during cold exposure of rats contributes to the increased metabolism of alcohol in these animals.

Treatment of the rats with perchlorate produced a marked hypertrophy and hyperplasia, an increased vascularity and a profound reduction in the amount of follicular colloid of the thyroid gland. Wyngaarden, et al (108) noted that treatment with perchlorate for 17 days resulted in hyperplastic, colloid-depleted, low iodine goiters. In the present experiment the thyroids were clearly hyperplastic and colloid-depleted, indicating hypo function after five days of treatment. This was particularly pronounced in cold-acclimated animals. Alcohol metabolism in the latter group was, however, similar to that in the non-treated controls, although the thyroid block seemed efficient. This might be interpreted as a confirmation of other observations concerning the relative dispensability of thyroid hormones in cold-acclimated animals. On the other hand when the rats were exposed to cold for five days only and their thyroid function blocked by perchlorate, the rate of alcohol oxidation was substantially reduced as compared to the untreated cold-exposed controls. It would appear, therefore, that a decrease in thyroid function of animals constantly exposed to cold could lower the overall metabolic activity of the liver, as determined by its capacity to oxidize alcohol. This would also be consistent with the increased requirements of thyroid hormones in rats constantly exposed to cold. Perchlorate treatment was

also responsible for a depression of the alcohol metabolism in rats kept at room temperature. In these animals, as well as in cold-exposed ones, the reduction was significant only for the first three hour period. It is also known that hypothyroidy of warm-acclimated rats is accompanied by decreased basal metabolic rate, following which the rate of metabolism of alcohol would be decreased as well as that of other substrates.

These results are in agreement with the more recent interpretations of the role of thyroid hormones in energy metabolism, according to which the level of thyroid function would exert a regulatory effect on energy metabolism within the zone of thermal neutrality or of comfort, increased levels of thyroid function would be necessary in the progressive establisment of non-shivering thermogenesis in animals exposed to cold, and would be relatively dispensable in cold-acclimated animals where they would exert only a supporting or "permissive" role or where their role in regulating the basal metabolism is exerted on an only insignigicant portion of the total metabolism.

In short, the effect of thyroid hormones on the metabolism of alcohol would be an indirect one, consecutive to their effect on the general metabolic rate.

GENERAL DISCUSSION AND CONCLUSION

The study of the metabolism of ethyl alcohol in rats exposed to cold environment was investigated by means of uniformly ^{14}C-labelled ethyl alcohol.

It is evident from the present study that in rats exposed to cold for a short period of time (five days), the rate of metabolism of alcohol was accelerated in contrast to their controls kept at room temperature. This increased metabolism was particularly pronounced during the first three hours following its administration, i.e. the period when most of the alcohol was oxidized. Furthermore, the rate of absorption of alcohol from the site of its administration and the rate of its removal from the blood was greater in cold-exposed animals than in rats maintained at room temperature. Forbes and Duncan (23) also reported an increase in the rate of alcohol removal from the blood of rats exposed to cold. In the present work, however, it has been shown that the observed increase in the rate of metabolism of alcohol in cold-exposed rats was not proportional to the rate of increase in general metabolism. The specific activity of respiratory $^{14}CO_2$ was lower throughout the duration of the experiment in animals exposed to cold than in their controls kept at room temperature.

In cold-acclimated rats, whether or not pretreated with alcohol, cold exposure also resulted in an increased rate of alcohol metabolism. Nevertheless, it would appear that the acclimation process is not responsible for the enhancement of alcohol metabolism, since a similar increase in alcohol metab-

olism was observed in rats exposed to cold for only a short period of time.

In cold-acclimated rats, alcohol pretreatment was accompanied by an enhanced rate of alcohol metabolism which was proportional to the increase in over-all metabolism. This was not observed in cold-acclimated rats or in rats exposed to cold for a short period of time when neither had been pretreated with alcohol.

In our experimental procedure we have measured combustion of ethyl alcohol - that is the over-all conversion of $^{14}C_2H_5OH$ to $^{14}CO_2$. This procedure enables one to determine the rate of its oxidation but tells nothing of the intermediate reactions. Since we have no direct evidence to explain why the relative metabolism of alcohol in alcohol pretreated cold-acclimated rats at the peak of alcohol metabolism compared favorably with that of the controls kept at room temperature, we can only assume that this phenomenon may be due to some modification of the metabolic pathways of alcohol.

It is of interest to note that the $^{14}CO_2$ elimination curve in the present study formed a straight line during the period when alcohol was being rapidly metabolized, followed by a hyperbolic curve when the amount of alcohol remaining in the body has dropped sharply. The length of the linear part of the elimination curve was conditioned by environmental conditions and by the dose of alcohol, being shorter in rats kept at room temperature than in cold-acclimated rats or in rats exposed to cold for a short period. It was increased in length by the larger doses of alcohol. It was reported

by Marshall and Owens (61) that the rate of metabolism of alcohol in mice is appreciably greater in the first hour than during subsequent hourly intervals. Forney, et al (24) confirmed Marshall and Owens (61) hypothesis, but found the early rapid rate of metabolism to occur during the first 30 minutes after administration. Kinard, et al (46, 47), however, could find no significant acceleration of metabolism in the mouse during the first hour following alcohol administration. At a very low level of blood alcohol, when alcohol dehydrogenase is not saturated, the alcohol concentration and the rate of utilization does not follow a straight-line relationship but an exponentional one, typical of first-order kinetics, as shown by Marshall and Fritz (62).

In the present study it has been shown that alcohol, when given in various doses, is metabolized at similar rates for as long as six hours following its administration. Identical results were reported by Jacobsen (42) and many others. Results contrary to ours were reported by Fazekas (21). The present results are also in contradiction with the reports by Vitale, et al (96) and Segovia-Riquelme, et al (81). Our results do not agree with those of Dontcheff and Porter (17), where alcohol administered to rats kept at room temperature only, was reported to be utilized in proportion to the amount of foodstuff oxidized at the same time, independent of the dose of alcohol administered. Our results are contradictory to the latter statement. We have demonstrated a dose dependence of the ratio of oxidation of alcohol to the total foodstuff utilization at the same time. These ratios, which we refer to as

the specific activity of $^{14}CO_2$ were different between the groups of animals given various doses of alcohol.

Even moderate doses of alcohol cause considerable cutaneous vasodilatation, thus preventing the normal cutaneous vasoconstriction upon exposure to cold. Central vasomotor depression probably plays a major role in the production of this peripheral vasodilatation, because the concentrations of alcohol found in the body fluids have little effect directly on blood vessels (21). The hypothalamic centers controlling heat production and heat conservation apparently exert their controlling influence upon temperature through the transmission of sympathetic impulses to the cutaneous vessels and pilomotor muscles via the autonomic nervous system. The depression of hypothalamic centers through high levels of alcohol might thus result not only in the abolition of responses from the exteroceptors but also in the depression of autonomic and endocrine components. In animals given high doses of alcohol and exposed to cold, the decrease in body temperature could result from continuous vasodilatation. Additionally there might be some central effect. These processes, if operating in the rat, may explain the practically unchanged specific activities of $^{14}CO_2$ during the first six hours-period when most of the alcohol was present in the body - following administration of high doses of alcohol to cold-exposed rats.

Although the QO_2 of liver is substantially increased in partially hepatectomized, cold-exposed rats compared to their room temperature controls (102), we were unable to notice any detectable changes in alcohol oxidation rate in cold-exposed

rats whether or not submitted to partial hepatectomy.

Evidence has been given that the thyroids (8, 14, 15, 82, 84) and catecholamines (56) play an important role in cold-thermogenesis of homeotherms. Since both of these hormones are affected by alcohol administration, we investigated their possible role in affecting the rate of alcohol metabolism in animals exposed to cold and found that a single administration of an adrenergic blocking agent, phenoxybenzamine, immediately prior to the study of alcohol metabolism, produced a noticeable decrease in the oxidation rate of alcohol in rats exposed to cold. This reduction was even more pronounced when cold-exposed rats were pretreated with phenoxybenzamine from the onset of cold exposure, i.e. for five days prior to alcohol administration. It is not possible to assert which one of the catecholamines is responsible for the increased metabolism of alcohol in cold-exposed rats, since phenoxybenzamine is not specific for either adrenaline or noradrenaline.

It is not surprising to see a decreased alcohol metabolism in rats exposed to cold and treated either once or daily with phenoxybenzamine during this cold exposure. Phenoxybenzamine by virtue of its non-competitive blockade of the adrenergic mediator prevents access of the mediator to the receptor and thus may inhibit an increased mobilization of energy substrates normally occurring upon exposure of homeotherms to cold. As a consequence of this depression, the normally enhanced metabolic rate may be lowered. Therefore, we may assume that the increased metabolism of alcohol in cold is not directly related to the action of catecholamines, but may be associated with an increased

general metabolism, in which the catecholamines play important roles.

From the parameters of the experiment dealing with the thyroid blockade it seems reasonably certain that thyroids contribute toward the increased metabolic rate of alcohol in animals exposed to cold. This was especially visible in rats exposed to cold and pretreated with perchlorate from the beginning of cold exposure. Cold-acclimated rats, pretreated with perchlorate for similar length of time, however, had alcohol metabolic rates similar to the non-treated, cold-acclimated rats. This discrepancy in the thyroid block between cold-acclimated rats and those exposed to cold for a short period of time could be accounted for by several observations concerning the relative dispensability of the thyroid hormones in cold-acclimated animals.

It is evident from the present study that neither of these factors can be considered wholly responsible for the observed increase in alcohol metabolism in rats exposed to cold. We are inclined to believe that a complex of physiological and biochemical factors probably contribute to this increase in metabolism of alcohol. The metabolism of alcohol is a complex phenomenon, thus it is not surprising to find that we are able to pinpoint only a few factors involved in its metabolism by rats exposed to cold.

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