A STUDY OF ALCOHOL TOLERANCE IN ANIMALS AND HUMANS

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A STUDY OF ALCOHOL TOLERANCE IN ANIMALS AND HUMANS

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EDWARD STEPHEN CESTARIC

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INTRODUCTION

The oxidation of alcohol[1] by the liver has been studied in many ways by a number of persons. In 1937, Lundsgaard (1) showed that alcohol disappeared very rapidly from the isolated liver, whereas with perfused hind limb muscle no oxidation of alcohol could be demonstrated. In 1937, Dontcheff (2) showed that the oxidation in the fasting white rat was dependent upon the stage of metabolism through which the rat was going. During the first stage, when primarily carbohydrate was being metabolized, the rate of oxidation of the alcohol was quite high. In the next stage, during which a major portion of the fat was being utilized, the rate was comparatively low. In the final stage, in which primarily protein was being metabolized, alcohol was again found to be oxidized at a rapid rate. In 1936, Rosovskaya (3) demonstrated that hyperthyroidism caused little acceleration of alcohol oxidation, and in 1939, Mirsky and Nelson (4) showed that it was the liver and not the pancreas which influenced the oxidation of alcohol. It was substantiated that the rate of alcohol utilization was apparent. In the eviscerated rabbit it was shown that alcohol was not utilized even if glucose or insulin was added, which indicated that muscle did not utilize the alcohol.

[1] The alcohol referred to throughout this work is ethanol unless otherwise specified.

LeBreton (5) showed that when fasting dogs were given small doses of alcohol, about ninety per cent of the oxygen consumed was accounted for by the oxidation of the alcohol.

Alcohol tolerance, as viewed by Newman and Lehman (6) and Newman and Card (7) is primarily a tissue tolerance whereby the cells of the central nervous system acquire the ability to function effectively in the presence of alcohol. Later in 1941, Newman (8) further demonstrated this by habituating dogs to alcohol. He then gave test doses of alcohol to habituated as well as to non-habituated dogs. He found that the former showed a markedly greater ability to control their neuro-muscular apptitudes. The habituated animals "sobered up" more rapidly despite the fact that their blood alcohol levels fell at the same rate as that of the controls.

Richter (9) showed that rats preferred alcohol concentrations of one to six per cent in water; but when forced to take larger amounts of alcohol, they reduced their food consumption almost directly proportional to the caloric intake obtained from the alcohol. Since the rats grew and thrived under these circumstances, the results indicated that the alcohol may have replaced large amounts of food. Mitchell and Curzon (10) showed that the alcohol does possess a distinct energy food value. The energy of the alcohol is more sparingly utilized when carbohydrate food is simultaneously ingested. They postulated the oxidation of both alcohol and carbohydrate

is promoted by thiamine, which is essential for the metabolism of carbohydrate.

Possibly because of the fact that alcohol supplements the diet for certain necessary foods, the liver becomes damaged in excessive drinking. According to Iziri (11) alcohol has a specific toxicity for the liver. Later Gates (12) working with human subjects using the bromosulfalein test, showed that alcoholics, after prolonged and continuous drinking had liver impairment. Chaikoff, et al (13) working with dogs showed that severe fatty degeneration and even cirrhosis appeared in the liver where high protein diet with alcohol was given, and Lowry, et al (14) working with rats showed that with a low protein diet the substitution of twenty per cent alcohol for drinking water produced cirrhosis.

The foregoing experiments may lead one to suspect that the alcohol, itself, is the cause for the liver disorder. However, as pointed out by Jolliffe (15), Jolliffe and Jellinek (16), and Remington and Leitner (17) alcohol "per se" is not the cause. Inebriety and cirrhosis are definitely associated; but a combination of circumstances (e. g. alcoholism plus certain nutritional deficiencies)is required to produce liver damage.

HISTORICAL

Scientists and law enforcement agencies have made considerable progress in the development of methods and special apparatus for the detection of intoxication. That this is a fact, may be concluded from a statement from the F.B.I. Law Enforcement Bulletin of April 1, 1938, p. 13, "Scientific investigators have pointed out and repeatedly verified the fact that the concentration of alcohol in the body fluids is one of the most reliable and objective criteria of intoxication. Many of these investigators have determined the relationship between brain alcohol concentrations and concentrations in the spinal fluid, urine, saliva, and breath, so that the degree of alcoholic influence can be evaluated closely when the concentration of alcohol in the breath or in the body fluids is known." However, the methods for the detection of intoxication (Drunkometer, Intoximeter, and the Alcometer)(27) still provide no satisfactory explanation as to why some individuals are capable of consuming more alcohol than others before reaching the same degree of intoxication. The phenomenon of "tolerance" is quite complicated. Both the psychological factor involving the state of mind in which he may be and the physiological factor involving the type and amount of food consumed prior to alcohol intake may play an important role in so-called "tolerance."

Even with these complicating factors, certain individuals must consume more alcohol than others in order to reach the same degree of intoxication or blood alcohol levels. This is evident even under comparable conditions of alcohol consumption.

EXPERIMENTAL

Investigations by Dontcheff (2), Rosovskaya (3), and Mirsky and Nelson (4) showed rather conclusively that the liver is an important organ in the metabolism of alcohol. For these reasons in the present investigation it was decided to study the liver to determine whether there is any correlation between its function and tolerance of an individual towards alcohol.

In conjunction with this the alcohol concentrations of the blood, liver, and kidney were determined in order to ascertain any significant difference in the ratios of alcoholic concentrations of these tissues for the tolerant and non-tolerant animals.

In order to make a preliminary investigation by which the liver promotes "tolerance" in certain individuals and not in others, the latter part of this experiment was carried out using Quick's hippuric acid function method. It was hoped that a correlation might be found between the capacity of the liver to form hippuric acid and the capacity to metabolize alcohol. In this part of the experiment, "tolerant" and "non-tolerant" human subjects were chosen from individuals who had participated in previous experiments. (24) The amount of hippuric acid formed by the subjects before and after the administration of alcohol was used as the basis upon which the human liver function was determined.

To be familiarized with the procedure for the determination of alcohol concentrations of solutions by the modified Nicloux method, (18) a number of trial determinations were made. The modified Nicloux method involves the distillation of the alcohol from a Kjeldahl flask through a steam trap into a Liebig condenser. The distillate is collected in a test tube containing an acid-dichromate[2] mixture, boiled for fifteen minutes to ensure complete oxidation, and subsequently titrated with sodium thiosulfate using one per cent starch solution as an indicator. The reactions involved may be represented as follows:

1) $2K_2Cr_2O_7 + 8H_2SO_4 + 3C_2H_5OH \longrightarrow 3CH_3COOH + 2Cr_2(SO_4)_3 + 2K_2SO_4 + 11H_2O$

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

3) $3I_2 + 6Na_2S_2O_3 \longrightarrow 3Na_2S_4O_6 + 6NaI$

Determination of Alcohol in Water-Alcohol and Blood-Alcohol Solutions of Known Concentrations

A number of known water-alcohol solutions were prepared for the preliminary determinations. This was done first by redistilling alcohol four times and determining the final concentration of the solution by the use of a calibrated specific gravity bottle[3], and comparing to water at $20^\circ/20^\circ C$.

[2]The acid-dichromate mixture consisted of five milliliters of concentrated sulfuric acid and five milliliters of the 0.434N potassium dichromate.

[3]The specific gravity bottle and all other equipment used were calibrated before used.

The specific gravity was then converted to per cent ethanol and found to be 93.46 per cent by weight. The alcohol was sealed in two ounce bottles until ready for use in preparing the known solutions. From this stock solution the known water-alcohol solutions were prepared and their concentrations determined. The exact composition of the solutions was determined bu the difference in the weight of the pipette before and after delivery:

$$\frac{\text{Wt. of the alcohol delivered} \times 93.46\%}{\text{Total wt. of solution}} = \% \text{ of alcohol by wt.w/w}$$

Errors in the determination may result from a number of scurces as indicated by Bennett (24).

The strength of the potassium dichromate solution was 0.434 N (18). Five milliliters of this solution was equivalent to twenty-five milligrams of alcohol if completely reduced. The sodium thiosulfate used was approximately 0.05 N. A blank titration was made to determine the exact amount of sodium thiosulfate equivalent to five milliliters of the 0.434 N potassium dichromate. The amount of alcohol oxidized was then calculated as follows:

A = ml. of $Na_2S_2O_3$ used in sample titration

B = ml. of $Na_2S_2O_3$ used in blank

$$\frac{B - A}{B} \times 25 \text{ mg.} = \text{mg. of alcohol in sample}$$

$$\frac{\text{Grams of alcohol} \times 100}{\text{Wt. of sample}} = \text{per cent alcohol by wt.}$$

Table I gives the results of the percentages of recovery.

TABLE I

DETERMINATIONS OF KNOWN WATER-ALCOHOL SOLUTIONS

Water samples of known alcohol concentration by weight were prepared by the method described in the experimental part of this work. The determinations were then run on these samples using the modified Nicloux method.

Sample No.	% Alcohol in known	% Alcohol in determination	Per cent Recovery
1	0.219	0.216	99.01
2	0.219	0.214	97.94
3	0.219	0.216	99.01
4	0.219	0.204	93.04
5	0.219	0.212	96.78
6	0.219	0.210	96.11
7	0.219	0.210	96.11
1	0.0685	0.0685*	99.99
2	0.0685	0.0675	98.58
3	0.0685	0.0674	98.37
4	0.0685	0.0680	99.33
5	0.0685	0.0675	98.51
6	0.0685	0.0677	98.88
1	0.124	0.121	97.61
2	0.124	0.121	97.61
3	0.124	0.120	96.68
4	0.124	0.122	98.14
5	0.124	0.110	89.05

* This determination did not reach exactly 100 per cent

After the water alcohol solutions were analyzed, a number of blood samples of known alcohol content were used to secure the necessary accuracy in preparation for the analysis of tissue samples of the rats. These results are tabulated in Table II.

TABLE II

DETERMINATIONS OF KNOWN BLOOD-ALCOHOL SOLUTIONS

Blood samples of known alcohol concentration by weight were prepared by the method described in the experimental part of this work. The determinations were then run on these samples using the modified Nicloux method.

Sample No.	% Alcohol in known	% Alcohol in determination	Per cent Recovery
1	0.0882	0.0854	96.73
2	0.0882	0.0854	96.62
3	0.0882	0.0852	96.51
1	0.0566	0.0556	98.11
2	0.0566	0.0556	98.11
3	0.0566	0.0552	97.49
1	0.0310	0.0294	94.68
2	0.0310	0.0299	96.45
3	0.0310	0.0297	95.81

Experimental Work on Rats

In this study the determination of the "tolerance" was made with twenty-four rats. The twenty-four rats were divided into three groups (10-10-4), all fed the same stock ration,

which contained the following ingredients:

yellow corn meal	32.50% by wt.
ground wheat	25.00% by wt.
white milk powder	22.50% by wt.
linseed oil meal	10.00% by wt.
alfalfa	6.00% by wt.
Brewer's yeast	3.00% by wt.
table salt	1.00% by wt.

Five per cent alcohol was fed to one group of ten; ten per cent alcohol was fed to the other group of ten, and the remaining four were used as controls being fed plain water. Each group consisted of an equal number of males and females. Accurate records of food and liquid consumption were kept on twelve of the rats (six males and six females, four from each group) for a period of 128 days (19). During the first week of feeding a definite loss of coordination and lower food consumption for those receiving alcohol were noted. However, as "tolerance" was gradually developed, their coordination returned to normal, and food and liquid consumption increased. Table III gives a tabulation of the average amounts of food and liquid consumed per one hundred grams of body weight.

TABLE III

LIQUID CONSUMPTION

The figures are given in milliliters of liquid consumed per 100 gram body weight per day per rat. The percentages shown in parenthesis were calculated by using the control animals as 100%

	Female Rats			Male Rats		
	Control	5%	10%	Control	5%	10%
	13.26	17.35	12.31	5.97	11.68	7.95
	9.01	16.46	14.70	5.83	14.75	8.91
Av.	11.135	16.91	13.51	5.90	13.21	8.43
		(150%)	(121.2%)		(223.8%)	(142.9%)

FOOD CONSUMPTION

The figures are given in milligrams of food consumed per 100 gram body weight per day per rat. The percentages given in parenthesis were calculated using the control as 100%

	Female Rats			Male Rats		
	Control	5%	10%	Control	5%	10%
	6.57	4.48	4.74	4.43	5.69	4.85
	6.53	6.71	5.19	5.17	6.87	4.39
Av.	6.55	5.595	4.96	4.80	6.28	4.62
		(85.4%)	(75.6%)		(130.8%)	(96.2%)

After the required period of time for the development of tolerance elapsed, the rats were sacrificed. Three hours prior to sacrificing, each rat was given three grams of alcohol per kilogram of body weight by means of a stomach tube (20). (The alcohol was administered in 33-1/3% concentration.) At the end of the three hour period, the alcohol was assumed to be in a state of equilibrium throughout the body (20).

The metabolic rate of oxygen consumption of the liver was determined by the manometric Warburg technique. Before the animals were sacrificed the Barcroft-Warburg vessels were partially prepared by pipetting two milliliters of Krebs-Ringer Phosphate solution (pH 7.4) into the larger portion of the vessel[4] (21,22,23). The vessels were then set aside until the tissue slices were prepared. This procedure was essentially as follows: Immediately after the animal was sacrificed, the blood was withdrawn and placed in beakers containing sodium fluoride and sodium oxalate (a preservative and an anticoagulant) and refrigerated. The sodium fluoride and sodium oxalate was a one to one mixture, approximately one milligram of the mixture being used for every milliliter of blood. The mixture was wetted down with a few drops of distilled water and spread along the sides of the beaker and set to dry. The

[4] The potassium hydroxide was not put in at this time to eliminate any possibility of contamination of the tissue by placing it in contact with the potassium hydroxide.

kidney and liver were then removed and frozen until further use. For the Warburg determination the middle lobe of the liver was used.

The tissue slices were prepared by cutting cross-section areas of the liver approximately one centimeter in diameter and placing them between two microscope slides which were frosted by rubbing with emery cloth. A razor blade was then passed between the top slide and the tissue to obtain slices of about 0.2 millimeters thickness. The slices were weighed accurately to about 100 milligrams per vessel. The weighing was done as quickly as possible without suspending the slices in any liquid to prevent diffusion of the alcohol and were transferred to the Warburg vessel. The elapse of time between slicing of the tissue and placing them in the Warburg vessel was approximately ten to fifteen minutes. Two-tenths milliliters of 30% KOH was pipetted into the center well and small strips of filter paper were placed into the well to increase the absorption area. They were then attached to the manometers and gassed with oxygen. After the gassing was completed, the manometers were placed on a constant water bath for ten minutes to attain constant temperature throughout the system. When constant temperature was attained the stopcock was closed and the shaking begun. The shaking was continued for one-half hour with readings being taken at ten minute intervals. A thermobarometer was prepared in exactly the same manner except that the tissue slices were omitted. Dry weights of the liver were

obtained by weighing accurately 300-500 milligrams of the tissue slices and drying them to constant weight between 105° and 110° overnight.

It was found that rats which received the 10% alcohol solution had the lowest oxygen uptake, whereas those receiving water had the highest. The results are shown in Table IV.

TABLE IV

OXYGEN UPTAKE (Q_{O_2})

Q_{O_2} is expressed as oxygen uptake per milligram of tissue per half hour. The percentages given in parenthesis were calculated using the control as 100% uptake.

Female		
Control (2 animals)	5% (3 animals)	10% (3 animals)
-2.70 ± 0.130 (100%)	-1.89 ± 0.895 (70.0%)	-1.64 ± 0.390 (60.7%)
Male		
Control (2 animals)	5% (4 animals)	10% (4 animals)
-2.18 ± 0.152 (100%)	-1.96 ± 0.324 (89.9%)	-1.60 ± 0.285 (73.4%)

The alcohol concentration of the liver, kidney, and blood was also determined by the modified Nicloux method (18). In the preparation of the tissue for the determination, a known weight of the tissue was sliced into small pieces. These were then transferred quantitatively to a Waring Blender and macerated with a minimum amount of water. When the maceration was complete, the tissue homogenate was again quantitatively transferred to twenty-five milliliter volumetric flasks and brought to volume with distilled water. The foregoing was the method used for the liver and kidney. However, for the blood, a measured volume was transferred to ten milliliter volumetric flasks and brought to volume with distilled water. The average amount of blood extracted from the rats was about seven milliliters. Aliquots of the prepared tissues were then used for the determination. Because of the small quantity of sample available, it was necessary to change the concentrations of the reagents. Instead of using five milliliter samples, three milliliter samples were used and the strength of the potassium dichromate was reduced to 3/5 (or 0.261 N); likewise the sodium thiosulfate was reduced to approximately 0.03 N. Therefore five milliliters of the solution was equivalent to fifteen milligrams of the alcohol. The calculations were carried out in the usual manner -

$$\frac{B - A}{B} \times 15 = \text{mgs. of ethyl alcohol}$$

$$\frac{\text{Grams of alcohol} \times 100}{\text{Wt. of sample}} = \text{per cent of alcohol}$$

Table V gives the results of the alcohol concentrations of the tissues of individual rats and Table VI the average of each group.

TABLE V

ALCOHOL CONCENTRATION OF TISSUES

The concentrations are given in percentage of alcohol by weight in the tissues.

No.	Blood		Liver		Kidney	
			5% ANIMALS			
			(Female)			
1	0.0724	0.0718	0.0288	0.0226	0.146	0.134
3	0.0025	0.0028	0.113	0.108	0.159	0.169
1a.	0.0772	0.0791	0.192	0.194	0.133	0.142
2a.			0.148	0.143	0.124	0.108
3a.	0.0279	0.0269	0.051	0.0556	0.0495	0.0498
			(Male)			
2.	0.0264	0.0262	0.1057	0.113	0.148	0.154
4.	0.0113	0.0159	0.0399	0.0416	0.114	0.111
7a.	0.110	0.109	0.114	0.109	0.122	0.116
8a.	0.109	0.0989	0.0546	0.0608	0.0911	0.0833
9a.	0.0255	0.0361	0.114	0.110	0.124	0.136

TABLE V (Continued)

No.	Blood		Liver		Kidney	
			10% ANIMALS			
			(Female)			
5.	0.0706	0.0706	0.0458	0.0414	0.139	0.149
7.	0.0950	0.0939	0.1700	0.158	0.285	0.285
4a.	0.0883	0.0789	0.162	0.170	0.335	0.340
5a.	0.0149	0.0143	0.0688	0.0660	0.104	0.099
6a.	0.0179	0.0174	0.0262	0.0209	0.0951	0.100
			(Male)			
6.	0.0202	0.0192	0.100	0.101	0.0898	0.0864
8.	0.0187	0.0196	0.0984	0.104	0.0694	0.0743
10a.	0.167	0.168	0.135	0.147		
11a.	0.0237	0.0240	0.0184	0.0224	0.0873	0.0927
12a.	0.0701	0.0703	0.148	0.150	0.154	0.148
			CONTROL ANIMALS			
			(Female)			
9.	0.0975	0.0960	0.105	0.106	0.151	0.148
11.	0.0873	0.0884	0.0225	0.265	0.321	0.310
			(Male)			
10.	0.0279	0.0284	0.0790	0.0808	0.0927	0.0859
12.	0.0718	0.0718	0.0387	0.0329	0.0287	0.0335

TABLE VI

ALCOHOL CONCENTRATION OF TISSUES

The data in this table was obtained by determining the average alcohol concentration for the males and females from Table V, then determining an over all average of each group. The concentrations are given as percentages by weight.

	Blood	Liver	Kidney
	CONTROL ANIMALS		
Female	0.0923 ± 0.01	0.1755 ± 0.11	0.2258 ± 0.14
Male	0.0500 ± 0.02	0.0579 ± 0.04	0.0602 ± 0.05
Average	0.0712 ± 0.02	0.1167 ± 0.08	0.1430 ± 0.10
	5% ANIMALS		
Female	0.0453 ± 0.07	0.1056 ± 0.16	0.1215 ± 0.09
Male	0.0559 ± 0.12	0.0862 ± 0.09	0.1200 ± 0.05
Average	0.0506 ± 0.10	0.0959 ± 0.13	0.1208 ± 0.07
	10% ANIMALS		
Female	0.0563 ± 0.09	0.0929 ± 0.17	0.1934 ± 0.28
Male	0.0601 ± 0.14	0.1025 ± 0.11	0.0753 ± 0.17
Average	0.0582 ± 0.12	0.0977 ± 0.14	0.1344 ± 0.26

Experimental Work on Human Subjects

The third part of the experiment was done with human subjects. The purpose was to ascertain whether there was any correlation between the formation of hippuric acid and the degree of "tolerance" after the administration of alcohol. From previous tests (25) a number of individuals who were known to have a high "tolerance" and those of a low "tolerance" were selected. Quick's liver function test (24,26), for the formation of hippuric acid, was used as the basic criterion. This test is based upon the synthesis of hippuric acid by the liver from benzoic acid and aminoacetic acid. The hippuric acid is excreted in the urine normally at a nearly constant rate. First a normal liver function test was carried out with each subject. Then after several days had elapsed the subject was allowed to ingest a certain amount of alcohol in the morning after a light breakfast. This was done by administering sufficient alcohol[5] to the individual until his blood content rose to about 0.10%. The blood alcohol concentration was determined on the Alcometer[6] (25). Breath tests were taken at intervals and when the desired concentration was reached, Quick's test was again performed and the results obtained with the two types of individuals compared. The data are shown in Table VII.

[5]Kentucky Tavern. Bottled in bond by the Glenmore Distillers Company, Owensboro, Kentucky.

[6]Alcometer, manufactured by Alfred Bicknell Associates, Cambridge, Mass.

TABLE VII

QUICK'S TEST--HUMAN SUBJECTS

TOLERANT

	Subject	Grams hippuric acid excreted before	after	Difference grams	percent	Ounces alcohol consumed	Per cent alcohol in blood
	8	4.773	1.01	-3.763	78.8	12.0	.110
	9	6.281	1.261	-5.030	80.1	12.0	.099
	10	2.845	0.464	-2.390	84.0	13.5	.096
	22	5.047	0.590	-4.457	88.3	9.0	.083
	1	4.448	2.914	-1.534	34.4	12.0	.108
				-3.435 ± 2.6	82.8 ± 5.8	11.7 ± 2.1	.099 ± .03

NON TOLERANT

	4	2.40	1.125	-1.275	53.1	10.5	.100
*	5	1.030	0.329	-0.701	67.1	10.5	.110
*	11	4.150	0.765	-3.385	81.5	9.0	.096
	12	2.185	0.722	-1.436	65.7	10.5	.120
	16	1.987	0.483	-1.504	75.6	9.0	.096
*	19	2.380	1.948	-0.432	18.1	9.0	.100
	20	3.354	0.705	-2.649	78.9	9.0	.115
	21	2.456	0.479	-1.977	80.5	7.5	.102
	14	3.60	1.687	-1.913	53.1	10.5	.101
				-1.486 ± .48	69.4 ± 11.1	9.9 ± 2.62	.106 ± .02

* These figures were omitted in calculating the averages for they fall out of the range of the standard deviation.

SUMMARY

1. A study of oxygen consumption of liver slices from rats previously on water, 5% ethanol and 10% ethanol showed highest values for group receiving water and lowest for the 10% ethanol group.

2. A study of the alcohol content of tissues from the above animals, three hours after administration of three grams of ethanol per Kilogram of body weight, showed such variations as not to permit definite conclusions.

3. A study of alcohol "tolerant" and "non-tolerant" subjects showed that the latter had a significantly greater capacity to form hippuric acid after the alcohol concentration in the blood reached approximately .10%.

DISCUSSION

The results shown in Table III pertaining to liquid consumption (water, 5% alcohol, and 10% alcohol) in this experiment agree with those obtained by Richter (9). The animals receiving the five per cent alcohol solution exhibited the greatest amount of liquid consumption, followed by those on the ten per cent level, and lastly by those on plain water. Moreover, food consumption also coincides with his results inasmuch as those on the ten per cent alcohol diet ate less than the controls. Because of the variability of those on the five per cent level, no definite conclusion can be drawn.

The metabolic rate of the liver, as determined by the manometric Warburg technique, seems to indicate that the control animals maintained the greatest oxygen consumption, followed by those on the five per cent diet, with the ones on the ten per cent diet the least. However, because of the lapse of time between the slicing of the tissues and the actual performance of the Warburg determinations, this may not be the actual case. At the time of slicing the tissues, it would appear that all of them had the same alcoholic concentrations since each animal was administered the same dosage of alcohol per 100 grams of body weight. However, during the lapse of time between slicing and the measurement of respiration, it

may be that, in the case of the animals that had developed tolerance, the tissues may have oxidized the alcohol almost to completion. Whereas in the non-tolerant animals a slower rate of oxidation occurred. Therefore, it is possible that after a period of time, the tissue with the slower rate of oxidation would appear to have the greatest rate of oxygen consumption in the Warburg apparatus.

From the over-all alcohol concentrations of the tissues as shown in Table V, there can be no definite conclusions drawn because of the variation in values found. However, using the average concentrations from Table VI and calculating their ratios, slight differences are noted. In Table VIII the concentration of the blood (from Table VI) was taken as unity, and the ratios of the alcohol concentrations of the tissues of their respective groups were calculated.

TABLE VIII

COMPARISON RATIOS

The ratios were obtained by using the data from Table VI and using blood as unity.

Blood	Liver	Kidney
	CONTROL	
1.0	1.64	2.01
	5% ANIMALS	
1.0	1.90	2.39
	10% ANIMALS	
1.0	1.68	2.31

Here it may be seen that the ratio of the alcohol concentration of the control's liver to the five per cent to the ten per cent is 1.64 : 1.90 : 1.68, respectively. The ratio at the five per cent level is greater than that of the ten per cent and the control is the smallest. Moreover, assigning a value of one to the concentrations of the tissues in the control animals (i. e. blood 0.714 = 1; liver o.1167 = 1 ; and kidney 0.1430 = 1), it may be seen that the respective ratios for blood, liver, and kidney in the ten per cent animals are 0.817 : 0.837 : 0.94 and 0.711 : 0.822 : 0.845 respectively for the five per cent animals. Hence there is a slight decrease in the alcohol concentrations-the controls being highest and the five per cent animals lowest. The data are shown in Table IX.

TABLE IX

COMPARISON RATIOS

The ratios were obtained by using the data from Table VI and using the control as unity.

Blood	Liver	Kidney
	CONTROL	
1.0	1.0	1.0
	5% ANIMALS	
0.711	0.822	0.845
	10% ANIMALS	
0.817	0.837	0.94

In the experimental work with the human subjects, there is a definite indication that the "tolerant" individuals were less capable of forming hippuric acid than the "non-tolerant" after the administration of alcohol. It may be postulated that the "tolerant" subjects are more concerned with oxidizing the alcohol than with the formation of hippuric acid from the benzoate, whereas the "non-tolerant" individual is primarily concerned with the formation of hippuric acid, and consequently, does not oxidize the alcohol as rapidly. It may be speculated that a "tolerant" individual has, as a primary function, "educated" his liver to oxidize the alcohol, and then to carry on its other tasks. In the case of a "non-tolerant" person the reverse appears to be true. Therefore, using this as a criterion, the hippuric acid liver function test "Quick's test) might be further examined for use as a medico-legal test to distinguish between "tolerant" and "non-tolerant" individuals.

The main disadvantage to the Hippuric Test for Medico-Legal purposes is that the time required for its determination is too long. However, there is a possibility that the Sulfobromophthalein (Bromsulfalein) test*, (28) which requires a much shorter time, might be investigated for this purpose.

*The time required to run this test depends upon the amount of dye injected. A 5 mg. dose requires from 45 to 60 minutes. A 2 mg. dose requires 20 minutes.

The principle of this test involves the injection of sodium sulfobromophthalein into the blood stream. The dye is removed by the liver and excreted in the bile within a short time after injection. The degree of dye retention in the blood is then measured by a colorimeter method. Inasmuch as this particular test was not investigated for this purpose in this paper, the author regrets the unavailability of any statistical data concerning such.

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