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THE DISTRIBUTION OF ALCOHOL IN THE TISSUES OF THE RAT

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In order to determine the distribution of alcohol in the tissues, we have devised a method which is quite simple and accurate.

The animal is killed and the tissues removed as rapidly as possible. The tissues are placed in weighed 150 ml. extraction flasks immersed in solid carbon dioxide. The flasks and tissues are weighed and the tissues covered with a solution of picric and tartaric acids. The samples are then placed in a refrigerator until the analysis can be made.

The alcohol is removed from the tissues by steam distillation using an apparatus fitted with a hashing knife attached to a motor. Any loss of alcohol is prevented by the use of a mercury seal. In this way the tissue may be hashed and the alcohol removed by steam distillation in the same apparatus hence avoiding loss of alcohol during manipulation. The distillate is received in a volumetric flask. The size of the flask depends on the amount of alcohol in the sample. The size of the flasks in our experiments varies from 25 ml. to 250 ml. and the amount of the distillate collected varies in volume from 20 ml. to 100 ml. After the distillate is diluted to volume an aliquot of from one ml. to 10 ml. is taken for analysis and transferred to the distillation flask such as is used in the analysis of blood for alcohol (Fish and Nelson, 1941). The aliquot is diluted to 10 ml. with distilled water and sodium tungstate and mercuric sulfate-sulfuric acid solutions are added as in the analysis of blood (L. C.). The mixture is distilled, five ml. of distillate being collected, and the alcoholic content determined as in the analysis of blood (L. C.).

Apparatus: The special apparatus for hashing the tissues and removing the alcohol by steam distillation is constructed in such a way that a three-hole rubber stopper which will fit the 150 ml. extraction flasks, carries a steam inlet, an outlet fitted with a trap leading to a condenser and the hashing knife. The steam inlet should reach well into the flask so that the steam is discharged below the surface of the liquid covering the tissues. The hashing knife is all metal and consists of a circular "saw toothed" knife on the end of a solid rod. The construction is such that a mercury seal prevents vapor loss. The steam outlet leading to a condenser

has a trap to prevent liquids from being carried into the distillate mechanically. The other apparatus used in this work is the same as described in the analysis of blood (l. c.).

Reagents: The picric-tartaric acid solution is prepared by adding one volume of saturated picric acid solution to an equal volume of a 20 per cent solution of tartaric acid. Other reagents necessary are the same as those given in a previous paper on the analysis of alcohol in blood (l. c.).

Calculations:

$$\frac{\text{ml. water blank} - \text{ml. titration}}{\text{ml. equivalent to one ml. of standard } K_2Cr_2O_7} \times 0.5 = \text{mg. of ethyl alcohol in aliquot of sample}$$

In order to check this and other methods used in this laboratory we have used the following procedure in the preparation of standard dilute alcohol solutions: Commercial absolute alcohol is placed in a clean and thoroughly dried 50 ml. distillation flask. A small piece of metallic sodium is added and the apparatus is assembled. A similar 50 ml. distillation flask is used as a receiver. The latter flask carries a drying tube containing anhydrous calcium chloride attached to the side arm. After the reaction with sodium is complete the material is distilled and the middle one third of the distillate is collected. The alcohol is transferred to a clean, dry weighing bottle for use.

A series of small, thin walled vials should be prepared having a capillary inlet. These are weighed accurately then slightly warmed. The tip of the inlet is placed in the alcohol and as the vial cools alcohol is drawn in. To completely fill the vial the alcohol in it is heated to boiling and the inlet placed again in the alcohol. Upon cooling the vial nearly fills with alcohol. The tip of the inlet is sealed and the vial again weighed. Standard solutions are prepared by breaking the vial under water and diluting the solution to volume in a volumetric flask.

Several experiments were made in order to check the accuracy of the method. In order to do this a known amount of alcohol was added to the picric-tartaric acid solution and treated like a tissue sample. Table 1 gives some characteristic results.

TABLE 1.

Sample	Mg. of ethyl alcohol	
	Actual	Found
1.	3.06	3.10
2.	3.06	3.08
3.	1.83	1.85
4.	1.83	1.84
5.	0.91	0.90
6.	0.91	0.91

In other experiments definite amounts of alcohol were added to tissue samples. About half of each tissue was left untreated with alcohol and analyzed as a blank. In table 2 some of the characteristic results are given.

TABLE 2.

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

A number of determinations were made on tissues from rats receiving no alcohol in order to determine the amount of substances normally present which appear as alcohol. Table 3 gives the average concentration of these substances determined as mg. of alcohol per 100 grams of tissue. For sake of comparison the values are given for one rat which was injected intraperitoneally with 1.4 grams of alcohol per kilo body weight and then killed after ten minutes.

TABLE 3.

Tissue:	Mg. % Alcohol Normal	Mg. % Alcohol Injected+	Distribution Ratio++ Normal	Distribution Ratio++ Injected+
Liver	1.00	146.0	20	66.4
Heart	2.90	185.0	58	84.0
Brain	2.61	188.0	52.2	85.4
Spleen	4:30	234.5	86.0	105.3
Kidneys	1.35	170.5	27.0	77.5
Pancreas	1.10	82.6	27.0	37.5
Stomach (Washed)[a]	3.64	146.0	72.8	66.4
Small Intestine (Washed)[a]	1.32	71.4	26.4	32.4
Muscle	0.80	108.0	16.0	49.1
Bone	0.50	68.8	10.0	31.3
Gonads	0.83	138.0	16.6	62.7
Lungs	1.76	158.0	35.2	71.8
Blood	5.00	220.0	100.0	100.0

+Received 1.4 grams of alcohol per kilo injected intraperitoneally. The tissues were removed for analysis 10 minutes after the injection.

++The distribution ratio is the ratio between the concentration of alcohol in the tissues to the concentration in the blood. For convenience the ratio is calculated on a basis of 100 mg. per cent of alcohol in the blood.

(a) The contents of the organs were washed out with distilled water and the organ dried with a clean towel.

The distribution of alcohol in the tissues after oral administration was studied. The rats were fasted for 24 hours and then given 2.5 grams of ethyl alcohol per kilo body weight by means of a stomach tube made from a No. 8 French style catheter. The rats were anesthetized lightly using chloroform in order to insert the stomach tube and administer the alcohol solution. The rats were all free of the influence of the anesthetic within three minutes. The alcohol solution used contained 0.25 grams of alcohol per ml.

A definite time after the administration of the alcohol a blood sample was drawn from the heart and the rat killed by chloroform. The tissues were removed and the alcohol content determined. Table 4 gives the distribution ratio calculated on the basis of 100 mg. of alcohol per 100 ml. of blood. The ratios are calculated as follows:

$$\frac{\text{Alcoholic concentration in tissues (mg\%)} \times 100}{\text{Alcoholic concentration in blood mg.\%}} = \text{ratio}$$

The ratios given are the average obtained from six rats.

TABLE 4.

Tissue:	Distribution ratio after one-half hour
Liver	68.7
Heart	41.8
Brain	21.1
Spleen	71.6
Kidneys	65.2
Pancreas	41.0
Stomach (Washed)+	1892.0
Small Intestine (Washed)+	133.0
Muscle	37.0
Bone	18.8
Gonads	46.9
Lungs	58.2

+ The contents of the stomach and intestine were washed out with distilled water and the organs dried with a clean towel.

SUMMARY

A method for the determination of the alcoholic concentration in tissues is described. This method prevents loss of alcohol during manipulation and is simple, accurate and rapid.

A method used in this laboratory to prepare accurately dilute standard solutions of alcohol is presented.

The concentration of substances in normal rat tissues reacting as ethyl alcohol has been determined and is compared with the concentration of alcohol in the tissues after the injection of alcohol.

Data are given on the distribution ratios of alcohol in tissues to the alcohol in blood 30 minutes after the oral administration of alcohol.

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LITERATURE CITED

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