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A STUDY OF PRESERVED BLOOD SPECIMENS TAKEN FOR ALCOHOL DETERMINATION

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From the beginning of our experience with blood alcohol determinations for evidence in legal cases we have been called upon for information concerning the change of alcohol content in blood specimens after they have been drawn.

Samples usually come to this laboratory in chemically clean glass vials stoppered with new corks and containing sufficient dry potassium oxalate to prevent clotting. These containers are obtained ready for use from this laboratory by county officials. However, many blood samples received have been contained otherwise, and often the temperature treatment of the specimens has not been uniform.

To have available information about the probability that samples at the time of analysis contained the same amount of alcohol as at the time of drawing we performed suitable determinations, the results of which are presented here.

Methods:—The method noted in the tables as "macro" is our standard procedure for determining alcohol content of blood. One cc. of whole blood, serum or plasma is mixed with 20 cc. of saturated picric acid and about 10 cc. of liquid is distilled off this into 10 cc. of potassium dichromate solution containing 2.1288 gm. per liter. Twenty cc. of concentrated sulfuric acid are added and the titration is continued according to Harger's (1) method using ferrous sulfate and methyl orange. The "micro" method is an adaptation of the dessication system of Abels (2) to the Harger titration. One-half cc. of blood is put on the rolled filter and suspended in a rubber stoppered flask containing 5 cc. of the dichromate solution used in the "macro" method and 10 cc. of sulfuric acid. This set is allowed to stand 12 hours at room temperature, after which it is opened for titration. Five cc. of water are added and it is titrated with Harger's red reducing fluid just as in the macro method.

Merck reagent grade chemicals were used throughout. All glassware was cleaned with sulfuric-chromate cleaning fluid and allowed to dry after a water wash and a distilled water rinse. All alcohol values used in the tables are averages of duplicate deter-

minations, of which no pair was allowed to vary more than 5 mgm. % without a recheck. Blood supposed to be kept chilled was warmed to room temperature long enough to allow measuring out at 20°-25° C. the sample used in analysis.

Procedure and Results:—Blood specimens received at this laboratory for alcohol analysis are stored in a refrigerator from the time they are received until the case for which they are evidence is disposed of. An examination was made of a few of these stored specimens picked at random and representing about one tenth of the total number on hand.

Table I. Alcohol Content Change in Non-Sterile, Oxalated Blood Specimens Kept Corked and Refrigerated for Varying Periods of Time

Sample	Original alcohol content mgm.%	Days in refrigerator	Alcohol content after aging mgm.%
1	211	55	175
2	150	16	155
3	279	120	241
4	278	6	245
5	134	90	119
7	160	175	27
8	242	30	275
9	152	28	170
10	197	21	250
11	65	21	73
12	318	6	300
13	135	10	134

Table I shows that these blood samples rarely give the original alcohol value after being stored for a period. Thirty to forty mgm. per cent variations can be expected. Number 7 is an exception; a few similar ones are noted later. Some of the slightest changes may be attributed to changes in physical state of the blood with aging.

To find whether loss of alcohol to the air in contact with the sample was a great factor in the changed values, blood samples were drawn from drinking persons. Half the blood was corked and refrigerated immediately; the other half was placed in a tube, plugged with cotton and left at room temperature. Table II shows that after twelve hours the open specimens at room temperature lost no alcohol, nor had the alcohol value in mgm. per cent increased because of water loss. The work represented by this table was done to validate actual blood alcohols taken for a coroner's case and stored as the cotton plugged samples. In one week only two open specimens had lost appreciable amounts of alcohol. Bacterial examinations on blood agar plates showed growth from only

two samples, and these samples had no alcohol decrease. The increase in U2 is not great enough to be significant.

Table II. Sample Divided and Given Different Treatment

Sample	Corked and refrigerated.* Alcohol, mgm.%	Cotton plugged at room temperature. Alcohol, mgm.%	Time second group aged.
C	167	169	12 hr.
B	130	125	12 hr.
H	86	86	12 hr.
U	74	83	12 hr.
H2	86	43	1 week
U2	74	83**	1 week
S	19	18**	1 week
H3	90	47***	1 week

* Determined 12 hr. after drawing.
** Staph. growth.
*** Cork stoppered.

It was suspected that aged blood specimens might accumulate non-alcohol reducing substances on standing or during the distillation. Therefore, simultaneous determinations were run using macro and micro techniques with the hope that dessication would give an answer. From Table III it may be seen that in several instances the analyses by the dessication method more closely approximate the original values than do the subsequent macro analyses. However, since there are also some wide discrepancies with the micro method no certain conclusion can be made.

Table III. Comparison of Alcohol Content by Two Methods on Blood Samples Aged in Refrigerator

Sample	Macro mgm. per cent	Micro mgm. per cent	Age, days	Rerun 2 mo. later		Original values mgm. per cent
				Macro mgm. per cent	Micro mgm. per cent	
1	241	275	120			279
2	226	235	124			242
3	119	130	60			134
4*	155	130	16	125	137	150
5	175	187	55			211
6*	146	117	20		12.5	154
7	245	283	6			278
8	57	68	16			70
9	215	244	22			225

* No growth by blood agar plate at time of last run.

Table IV is self-explanatory. With one exception the two methods give nearly the same values, probably indicating that distillation does not give rise to non-alcohol reducing substances. That H(s) had a negative culture doesn't mean no bacterial growth occurred in it.

It was desired to know what effect room temperature would

Table IV. Comparison of Alcohol Content by Two Methods on Samples of Blood Kept at Room Temperature

Sample	A		B			Alcohol consumed*
	Macro mgm. per cent	Micro mgm. per cent	Macro mgm. per cent	Micro mgm. per cent	Bacteria	
H(b)	86	—	43	44	Staph.	5 oz.
H(s)	86	90	60	47	Neg.	
U(b)	74	—	83	87	Staph.	5 oz.
U(s)	83	85	—	—		
S(s)	19	8	18	13	Staph.	0.7 oz.

* Pure alcohol equivalent of gin and whiskey consumed.
A was determined within 12 hours; B after 1 week.
(b) corked, oxalated blood.
(s) cotton plugged blood serum.

produce on the alcohol content of specimens previously stored in a refrigerator. Table V presents some data obtained under such circumstances. The inexplicable descrepancies noted in the previous tables prevail.

Table V. Samples Refrigerated for Varying Periods Then Stored at Room Temperature

Sample	Original alcohol values mgm. per cent Macro	Age, days	Alcohol content when removed from refrigerator mgm. per cent		Alcohol content after 1 week at room temp. mgm. per cent	
			Macro	Micro	Macro	Micro
1	211	102	175	187	171	171
2	5	1	5	----	5	----
3	150	16	155	130	125	137
4	279	120	241	275	239	273
5	278	6	245	283	237	----
6	134	90	119	130	92	90
A*	97				101	105

* Standard alcohol solution (97 mgm. per cent) used to check methods.

The question arose in connection with a court trial of how much alcohol bacteria could produce in blood in a given time. Since the occurrence of bacteria in specimens on hand showed no parallelism with rise or fall of alcohol content, a series of special innoculation experiments have been begun. Table VI presents the first of the

Table VI. Effect of Innoculation on Alcohol Values of Blood

	Alcohol content, mgm. per cent	
	Marco	Micro
Normal blood sample	5	0
Sample 1 week after innoculation with mother of vinegar.	30	4
Sample 1 week after inoculation with unidentified pigmented psychrophilic Gram negative bacilli.	12	0

series since the results of the macro and micro techniques disagree. Perhaps from this type of procedure the explanation of the variety of alcohol values obtained on any blood specimen at different times can be found.

RESULTS:— (1) Blood specimens preserved in the refrigerator have practically no change in alcohol content for the first two to three weeks. After longer periods of time a series of determinations on a specimen may show discrepancies including increase and decrease in no set order. (2) The alcohol content of blood kept open at room temperature for a day is not changed, provided excessive evaporation of water is not permitted. (3) Bacterial growth in blood specimens was not always accompanied by change in alcohol content. (4) An unidentified bacteria and mother of vinegar produce non-alcohol reducing substances detectable by the distillation technique but not by the dessication method.

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