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Correlation Between Postmortem Ethanol Levels in the Blood and the Testicle*

A Computerized Study of 633 Determinations*

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Summary. This study emphasizes the value of the presence of ethanol in the testicle. It proves particularly useful in cases where blood and urine are no more present in the body.

In 633 cases where blood or urine were still available, a highly positive correlation between the blood alcohol and the amount of alcohol present in the testicle could be demonstrated, thus confirming the research carried out in the Department of Legal Medicine as far back as 1943.

An attempt is further made to assess the possible influence of such factors as putrefaction, submersion or post-traumatic anemia on this correlation.

Key words: Ethanol level, in the testicle – Alcohol test, postmortem in the testicle

Zusammenfassung. Nach langjährigen Untersuchungen wird an 633 statistisch ausgewerteten Fällen die Korrelation zwischen Alkohol im Hodengewebe und im Blut mit $r = 0{,}92$ ermittelt. Auf die Einflüsse von Fäulnis, Wasserliegezeit oder posttraumatischem Blutverlust wird hingewiesen. Akuter Blutverlust brachte keine nennenswerte Veränderung. Bei chronischem Blutverlust ist nach Kaufmann et al. [25] mit einer Beeinträchtigung zu rechnen. Insgesamt ist die Methode geeignet, eine Alkoholbeeinflussung bei männlichen Leichen bis zu etwa 72 h Liegezeit zu überprüfen, wenn keine Blutwerte ermittelt oder sonstige Körperflüssigkeiten bzw. Organe mehr gesichert werden können.

Schlüsselwörter: Äthanolnachweis, im Hodengewebe – Alkoholbestimmung, postmortem im Hodengewebe

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When the blood alcohol test was introduced in Belgium unofficially already a few years before World War II, it soon became obvious that the situation differed entirely according to whether one was dealing with a living person or a corpse. In the first case, though venules were unknown at the time, blood sampling could be performed correctly if the necessary precautions were taken to avoid contamination. When dealing with a dead body, the situation is different. Blood sampling is inevitably a messy operation and there always remains a degree of uncertainty as to the cleanliness of the glassware in which it is being collected. From the start one of us (F. Thomas) therefore decided, for safety's sake, to carry out the analyses routinely on three or more specimens: blood from the femoral vein incised transversally in the groin, the nearby testicle and urine or, in the female, a fragment of muscle, and to compare the figures. The testicle which to a certain extent is protected from putrefaction by its firm capsule, the tunica albuginea, and which, moreover, is easy to remove, is particularly suitable for this type of procedure.

The wisdom of this precaution was confirmed from the very beginning (1941) by a case where the blood, which had been collected in a test tube, apparently contained 4 g‰ ethylalcohol, whereas the urine was totally negative, which was obviously impossible. Recently, a nearly identical situation served warning of the permanence of the risk.

The above described methodology has never been taken at fault through the years and, as already emphasized previously by one of us in three successive papers [10, 20, 37] can be considered as entirely reliable.

Analytic Procedure

Preparation of the Samples

Blood from the femoral vein and tissue from the testicle were analyzed immediately or, at the very latest, within 12 h after the postmortem. Preservation was insured at 4° C.

Blood samples were analyzed as such. The testis was bisected, and a cluster of seminiferous tubules was removed, cut into tiny pieces on a glass plate with a scalpel, and used for alcohol determination.

Alcohol Dosage

Titrimetric Method. From 1938 to 1942 only the classical Nicloux method was resorted to. It was replaced from then onward by that of Casier and Delaunois [9] which, in its turn, was in due course adapted to the standards of the official Belgian legal method [31]. In the latter the alcohol is distilled under low pressure and the vapor is carried by a weak air current. This vapor, obtained by dry distillation, is captured in an exact volume of a sulfochromic mixture, ethanol being thereby oxidized to acetic acid. The excess of dichromate is determined by iodometric back-titration. The alcohol content of the sample is calculated from the amount of reduced dichromate. It is expressed in gram ethanol per 1,000 ml blood or gram ethanol in 1,000 g of tissue (g‰).

For the exact analytic procedure, the apparatus, and reagents, we refer to the above mentioned edition of the *Moniteur Belge* [31].

The analyses were performed on 1 ml blood and 1 g of the respective tissues.

Gaschromatographic Method. The method now used is a modification of the procedure published by Heyndrickx et al. in 1961 and 1969 [22, 23]. It is based on the simultaneous addition of an internal standard to the sample and a protein precipitation, followed by a gaschromatographic separation and dosage of different alcohols.

Half a milliliter of blood (or 0.5 g tissue), 0.5 ml of a 10% sodium tungstate solution in water, and 0.5 ml of $^2/_3$ *N* sulfuric acid containing 0.15% tertary butyl alcohol (I.S.) are thoroughly mixed (by means of a vortex) for 30 s and afterward centrifuged at 9,000 rpm for 3 min.

One microliter of the liquid phase is injected in a gaschromatograph.

Technical Data

Gaschromatograph: Hewlett Packard F & M Scientific 5750 Research Chromatograph
Column length: 180 cm
Column diameter: internal: 3 mm, external: 5 mm
Stationary phase: Graphpac coated with carbowax 1500 (80/100 mesh)
Detector: Flame ionization detector (FID)
Temperatures: Column: 95° C, oven: 110° C, detector: 247° C, injector: between 145° and 160° C
Carriergas: N_2 (25 ml/min)
Recorder: Hewlett Packard 7128 A, chart speed: 0.25 in./min.

This method permits separation of different alcohols (f.i. methanol and ethanol) and quantitative dosage by relative peak-area comparison with the internal standard.

Since 1962 titrimetric results have been controlled on each sample by the above described procedure.

Water Content

A certain amount of blood and thinly minced tissues (± 1 g) was weighed analytically on a tared watch-glass and dried in an electric oven at 60 to 70° C for 24 h. The whole was forthwith cooled to room temperature in a dessicator.

After weighing, the drying procedure was repeated until a stable dry weight was obtained. The water content was calculated out of the difference between the wet and dry weights.

In 1957 we decided to take advantage of the improved chemical methods for alcohol analysis now at our disposal to take this research up again this time on a large scale. The data presented in this investigation cover a period of approximately 23 years.

Blood was systematically sampled and the testicle removed in 633 bodies (urine was also analyzed in 442 of the cases, but the results will be dealt with in another paper).

For each case the testicle alcohol was plotted vs. the alcohol concentration of the blood samples and the ratio was calculated.

To detect possible variations in the relationship between blood and testicle alcohol values, our material was divided into three groups:

Group A ($n = 114$) assembles the cases where there had been no or only little loss of blood.

Group B ($n = 430$) includes the traumatic cases (mainly traffic accidents) having entailed heavy loss of blood.

Group C assembles 89 cases of drowning. They were kept apart because an unknown amount of hemodilution might have occurred and, moreover, because putrefaction had usually set in before the body had surfaced [8, 19, 27].

In each case we endeavored to determine the time interval between the occurrence of death and the autopsy. As far as groups A and B were concerned there was surprisingly little difference from one case to another (median interval: 24 h). As was to be expected, this was not the case with group C where the intervals are much more diverse, the median interval being 72 h.

Results

Distribution of the Alcohol Concentrations

In Tables 1 and 2 the different descriptive distribution parameters are listed for the total population under study and separately for the three groups. The alcohol concentrations are expressed in g‰.

Table 1. Distribution of the blood alcohol concentrations

Group	Number of cases	Mean	SD	Median	5th perc.	95th perc.
A	114	1.53	0.99	1.53	0.06	3.24
B	430	1.61	0.86	1.71	0.05	2.80
C	89	1.59	0.96	1.80	0.04	2.87
Total	633	1.60	0.90	1.69	0.06	2.86

Table 2. Distribution of the testicle alcohol concentrations

Group	Number of cases	Mean	SD	Median	5th perc.	95th perc.
A	114	1.50	0.99	1.51	0.06	3.12
B	430	1.54	0.87	1.55	0.05	2.82
C	89	1.48	0.95	1.57	0.07	2.85
Total	633	1.52	0.90	1.55	0.06	2.85

Table 3. Distribution of the ratios testicle alcohol/blood alcohol

Group	Number of cases	Mean	SD	Median	5th perc.	95th perc.
A	114	1.08	0.73	1.00	0.60	1.45
B	430	1.01	0.69	0.95	0.54	1.42
C	89	1.21	1.37	0.95	0.52	2.75
Total	633	1.05	0.83	0.96	0.56	1.52

Table 4. Correlation between testicle and blood alcohol concentrations

Group	n	r	r^2	P	r'
A	114	0.94	0.88	<0.001	0.94
B	430	0.91	0.83	<0.001	0.90
C	89	0.89	0.79	<0.001	0.87
Total	633	0.92	0.85	<0.001	0.92

The distribution of the ratio: testis alcohol vs. blood alcohol is outlined in Table 3. The outspoken spread and asymmetry in the distribution of the parameters in group C (drowning cases) is conspicuous. In groups A and B the ratio approaches the unit.

Table 5. Linear regression coefficients

Group	*n*	a	b
A	114	0.06	0.94
B	430	0.05	0.93
C	89	0.08	0.88
Total	633	0.05	0.92

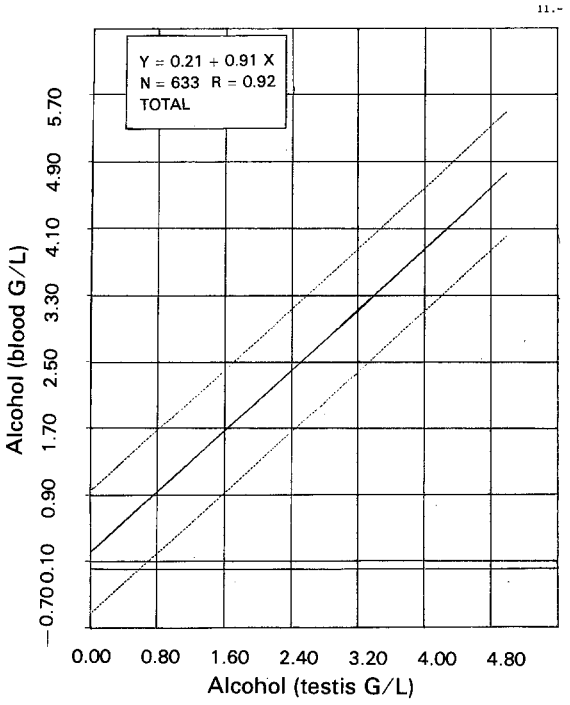

Fig. 1

Correlation Between Testicle Alcohol and Blood Alcohol

Table 4 illustrates the correlation coefficient (r) and the determination coefficient (r^2) of Pearson, with its accompanying significance level P. The correlation coefficient of Spearman (r') has also been calculated since it provides valid conclusions when the distributions diverge from the gaussian. A significant correlation was demonstrated for all three subgroups and for the total population.

Computation of the Regression Coefficients

In view of assessing the relation between the alcohol concentration in the blood and that in the testicle with more precision the regression parameters between both variables were calculated. A linear relation was postulated: $y = a + b \cdot x$. In this formula y expresses the testicle concentration and x the blood concentration. To verify the linearity the best fitting polynome of the equation $y = a + b \cdot x + c \cdot x^2$ was calculated from the data. No more variance of y could be predicted by this quadratic function. This confirms the validity of the linear model.

No significant difference could be put in evidence between the regression parameters of the three respective groups in our study (Table 5).

Computation of the Alcoholemia from the Testicle Alcohol Concentration

The correlation between the testicle alcohol concentration and the alcohol concentration in the blood being established, it was now investigated in how far the testicle alcohol concentration reflected the alcoholemia. For this purpose, we constructed an inverse regression line with the blood concentration as dependent variable y (Fig. 1).

The 95th % confidence interval of the observations was plotted on the graphic (dotted line).

As, in our cases, the alcohol concentration distribution deflects to a certain extent from the gaussian, there ensues that these confidence intervals do not lend themselves to an absolute interpretation. They nevertheless give an idea of the range of the possible alcoholemias on the basis of a known testicle value.

Figure 1 (regression line for the entire group) informs us that an alcohol concentration in the testicle of, e.g., 1.6 g‰ corresponds to a mean alcoholemia of 1.7 g‰ and a minimum one of 0.9 g‰.

There is nevertheless a 2.5% probability that the blood concentration is even a little lower. Using an unilateral probability of 1/1,000 and rounding off to the safe side, the following rule of thumb which applies to the whole group will prove helpful: A testicle alcohol concentration of x g‰ corresponds to an alcoholemia which is not lower than $(x - 1.3)$ g‰.

Discussion

It is obvious from the above that there exists a constant correlation ($r = 0.92$ with $P < 0.001$) between testis and blood alcohol values. The ratio approaches the unit ($\hat{x} = 1.05$ and $x_{med} = 0.96$).

The testicle therefore lends itself optimally to the assessment of the alcoholemia existing at the time of death in those cases where blood was not available any more because of exsanguination following heavy hemorrhage or because of decomposition. This confirms fully the early findings (1953) of one of us [20, p. 25].

A recent paper by Zoroastrow and Avramenko [42], two Russian authors, reports practically the same results, their ratio being 0.95.

We have checked the respective ratios separately for our three groups.

Group A: $\hat{x} = 1.08$ median 1
Group B: $\hat{x} = 1.01$ median 0.95
Group C: $\hat{x} = 1.21$ median 0.95

No statistically significant differences can be demonstrated between the mean ratios.

The ratios are much more dispersed in group C, i.e., that of the drowning cases: 5th percentile 0.52 and 95th percentile 2.75 to be compared with the total group 5th percentile 0.56 and 95th percentile 1.52. These broader fluctuations are easy to explain. First of all, more time elapses between the occurrence of death and the

recovery of the body for postmortem examination (72 h on the average instead of 24 h). Putrefaction has thus set in. Furthermore, we must keep in mind the possibility of hemodilution which often occurs in drowning.

Group A (nontraumatic causes of death) does not differ from group B (lethal trauma). This tends to confirm that the so-called acute hemorrhage does not influence the alcoholemia [11, 32], whereas repeated, chronic hemorrhage accompanied by shock (e.g., due to a stomach ulcer), on the contrary, does [25]. In our B series survival was short.

If we consider the entirety of our cases, the spread of the ratios tends undeniably to be broad, i.e., 5th percentile 0.56 and 95th percentile 1.52 for a median value of 0.96.

We will, for the present, disregard the possible technical errors already recalled. Putrefaction is the main culprit (cf. group C). One of us, as aforesaid, showed that already after 48 h scattering of the figures was obvious [37].

It is well known that the water content of blood and tissues alike undergoes notable changes after death, with a resulting repercussion on their alcohol content. The water content usually diminishes, bringing about in its turn a decrease of the alcohol content. Less frequently, it is the opposite. The reader will find more information on the subject in the existing literature [1, 2, 6, 7, 18].

The authors suggest that the alcohol figures provided by any decomposed body should be correlated with the water contents of the respective fluids and organs.

Recent research [5] has established that no significant changes in the alcohol values will occur if a body has been kept, even as long as 5 days, at a temperature of 4° C.

Since the alcohol ratio blood/testicle comes close to the unit, this implies that the same is true for the water content. We checked this analytically and found for the testicle water content a mean value of $85.6 \pm 1.15\%$ with a reliability coefficient of 99%. This figure is the same as that reported recently by Bilzer and Kühnholz [3]. This shows that the water content of the testicle is much more stable than that of the blood. The latter can undergo quite broad variations [1, 2] which is easy to understand. The testicle is completely isolated from the outside world by its strong protective covering, the albuginea.

We will now consider the possible postmortem diffusion of the alcohol present in the stomach as cause of error. This can undoubtedly happen as Schleyer has confirmed experimentally [35]. Many authors, while not denying this possibility, maintain that it does not have any marked influence on the alcohol concentration of the blood present in the neighboring heart [34, 36]. We do not entirely agree with the latter optimistic view. A few cases have been reported where a person, having died after emptying a whole bottle of gin and the alcohol having diffused slowly upward after death, through the diaphragm, the heart blood contained an enormous amount of alcohol, entirely out of proportion with that found at the level of the femoral vein.

Some authors stress the different alcoholemias according to the region of the body where the blood was collected and that even in the absence of putrefaction [24]. There is disagreement on the matter [12]. There is a general consensus to the effect that the femoral vein is the safest spot for sampling. It is generally accepted that decomposition can lead to bacterial neoformation of ethylalcohol. There is,

however, considerable divergence of opinion as to how much. We prefer, for the present, to keep at a safe distance of this hornets' nest [4, 17, 30, 39]. Be it only reminded that this possible set-back can be avoided by keeping the body in a refrigerator at 4° C.

As to the possible break-down of the alcohol, be it of bacterial origin or through extrahepatic alcohol dehydrogenase, opinions differ. In any case, it seems unreasonable to ascribe any practical implication to a suchlike phenomenon [32]. The ratio will obviously differ according to whether death occurs during the absorption phase or later on in the elimination phase [29]. This is, of course, a general rule. We refer to a recent paper of Kühnholz and Bilzer [28].

From the aforesaid we are entitled to conclude that the excellent correlation between blood and testicle alcohol provides a reliable means of appraising the result of the blood analysis. Even in the absence of blood in the body, one is entitled to infer the approximate alcoholemia at the time of death from the testicle alcohol. For this purpose, we have computed a regression line where the dependent variable y expresses the alcoholemia.

The y-line $y = 0.21 + 0.91x$ holds for the entire group. The expected value of blood concentration is situated statistically in between relatively broad limits, with the medico-legal implications thereof. There is no doubt that, for medico-legal purposes, the testicle alcohol is much more reliable than the level disclosed by the analysis of the urine alcohol, which can vary for many reasons too well known to be gone into [40]. If a 1/1,000 possibility of error is considered the following rule can be put forward.

A testicle alcohol concentration of x g‰ corresponds to an alcoholemia which is not lower than $(x - 1.3)$ g‰ (this being the purely statistically extreme lower limit value). To take a practical example, if the testicle alcohol is 2 g‰ we are only entitled, for safety's sake, to infer from this figure an alcoholemia of 0.7 g.

If putrefaction is advanced it seems advisable to correlate the alcohol values with the water content of blood and testicle. To date we have done this a number of times with encouraging results: the water content of the testicle remains practically unchanged.

Just as organic fluids: urine [14], bile [38], cerebrospinal fluid [16], seminal vesicle content [33], corpus vitreum [13, 15, 21] and solid tissues, such as brain, kidney, muscle, bone marrow, etc. [20, 26, 41] lend themselves to the determination of their alcohol content, so does the testicle, as reported by one of us [37, p. 36] as far back as 1951 on the basis of the analysis of 43 cases.

It can even be said that, for that purpose, it is privileged because of its resistance to putrefaction which is due to its tough fibrous covering. Moreover, it provides a reliable control value with regard to the alcoholemia, as its analysis will eventually permit to detect a doubtful analytic result or discover an accidental interchange of blood samples.

If it has been impossible to collect blood because of its absence consecutively to decomposition, the testicle value will still make it possible to assess approximately the alcoholemia.

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