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A New Perspective in the Estimation of Postmortem Interval (PMI) Based on Vitreous $[K^+]$*

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ABSTRACT: The relation between the potassium concentration in the vitreous humor, $[K^+]$, and the postmortem interval has been studied by several authors. Many formulae are available and they are based on a correlation test and linear regression using the PMI as the independent variable and $[K^+]$ as the dependent variable. The estimation of the confidence interval is based on this formulation. However, in forensic work, it is necessary to use $[K^+]$ as the independent variable to estimate the PMI.

Although all authors have obtained the PMI by direct use of these formulae, it is, nevertheless, an inexact approach, which leads to false estimations. What is required is to change the variables, obtaining a new equation in which $[K^+]$ is considered as the independent variable and the PMI as the dependent. The regression line obtained from our data is $[K^+] = 5.35 + 0.22$ PMI, by changing the variables we get PMI $= 2.58[K^+] - 9.30$. When only nonhospital deaths are considered, the results are considerably improved. In this case, we get $[K^+] = 5.60 + 0.17$ PMI and, consequently, PMI $= 3.92[K^+] - 19.04$.

KEYWORDS: forensic science, forensic pathology, postmortem interval, vitreous humor, potassium, regression

The relation between the increase of potassium concentration ($[K^+]$) in the vitreous humor and the postmortem interval (PMI) has been a topic of study for many years. Although numerous formulae in the literature correlate this relationship to a linear regression (Table 1), differences in technique, as well as factors of climate, transport, handling, and lack of complete data (9–13) make comparative studies of proposed equations impossible. Moreover, all proposed formulae obtained from the regression equation to estimate the PMI have been flawed by a serious and persistent error due to the fact that PMI has always been calculated by solving the equation with $[K^+]$ as the random, dependent variable, i.e., on the left hand side of the regression equation. However, we maintain that this approach is inexact, the precise method being to make an inverse prediction by changing the variables (14,15).

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The aim of this paper is to draw up a new formula based on this revised approach and we provide full data from our records to facilitate comparative studies. We also investigate the influence of urea and creatinine levels in the relationship between $[K^+]$ and PMI.

Material and Methods

We studied 201 samples from 164 deceased subjects (in 37 cases samples from both eyes were obtained simultaneously) received for autopsy in the Institute of Legal Medicine of the University of Santiago de Compostela, Spain. All cases were registered on an index card with the following information: case number, sex, age, medical care, cause of death, time of death, extraction time, and toxicological screening.

Samples were obtained by scleral puncture near the outer canthus using a 20 gage needle and a 10 mL syringe. Suction was applied gradually and slowly to withdraw all extractable vitreous humor according to Coe and Madea et al. (10,16).

Nontransparent specimens and those from newborn infants aged less than six months were considered unsatisfactory for analysis (16). Each sample was centrifuged at 3000 rpm for 10 min (5), and only the supernatant partis was used in order to avoid obstructing the fine tubing used in most current analytical instruments (11). The time between extraction and analysis was never more than 24 h, during which time samples were kept at 4°C.

Biochemical and toxicological parameters and analytical methods used are given below in Table 2. All results were obtained with a BM/747 (Boehringer Mannheim), except for alcohol levels, which were determined with a 5890-Series II (Hewlett Packard). The PMI in hours was expressed in the decimal system and statistical analysis carried out using SPSS 9.0.1 for Windows™ applying simple linear regression.

Results

Samples from corpses whose time of death could not be established to within ±15 min were excluded.

A total of 201 samples, from 127 men and 37 women, were used. All data are shown in Table 3. The minimum value of PMI was 1.00 h and the maximum 40.45 h, with an average of 11.00 h and a standard deviation of SD = 7.71.

We obtained a linear regression formula with PMI as the independent variable and $[K^+]$ as the dependent. No significant dif-

TABLE 1—*Traditional formulae for determining PMI.*

Author–Year	Equation Obtained*	Formula Proposed†
Sturner 1963 (1)	$y = 0.14x + 5.6$	$PMI = 7.14[K^+] - 39.1$
Adelson et al. 1963 (2)	$y = 0.17x + 5.36$	…
Hansson et al. 1966 (3)	$y = 0.17x + 8$	…
Coe 1969 (4)	$y = 0.332x + 4.99$ ($x < 6h$)	…
Coe 1969 (4)	$y = 0.1625x + 6.19$ ($x \geq 6h$)	…
Adjutantis & Coutselinis1972 (5)	$y = 0.55x + 3.14$	…
Stephens & Richards 1987 (6)	$y = 0.238x + 6.342$	…
Madea et al. 1989 (7)	$y = 0.19x + 5.88$	$PMI = 5.26[K^+] - 30.9$
James et al. 1997 (8)	$y = 0.23x + 4.2$	$PMI = 4.32[K^+] - 18.35$

* $y = [K^+]$ mmol/L and x = hours postmortem.
† PMI = postmortal interval in hours.

TABLE 2—*Analytical methods.*

Parameter	Analytical Methods
Potassium	Indirect potentiometry
Urea	Kinetic UV assay for Urea/Urea Nitrogen
Creatinine	Jaffá-Method taking into consideration the sample blank (twin mode)
Alcohol	Head space gas chromatography

ference arising from alcohol level was observed ($p = 0.803$), nor were there signicant differences between both eyes ($p = 0.914$).

All samples were classified into two groups: cases without known metabolic disturbance (Group A) and cases with some disturbance (Group B). Group A consisted of more usual forensic cases, rapid deaths, including sudden natural death, and traumatic death (accidental and suicidal). Group B consisted of those cases of protracted death, death involving hospitalization in Intensive Care Units, and cases of natural death from chronic illness. There were significant differences between the two groups ($p = 0.002$). Accordingly, 133 samples (group A) and 30 samples (group B) were analyzed giving $R = 0.838$ and $R = 0.685$ respectively. Lack of data excluded one case from inclusion in any group.

In order to increase the precision of these results we established critical levels of urea and/or creatinine according to Madea et al. (7); by using different values of urea and creatinine we found that best results were obtained by excluding cases with urea <30 mg/dL and creatinine ≥ 0.5 mg/dL. The linear regression and the proposed formula are shown in Table 4. The confidence intervals are given in Table 5.

Discussion

We propose a new and more precise formula to estimate the PMI from vitreous $[K^+]$. The difference between this and the many previously proposed formulae lies in changing the variables. We maintain that, according to the mathematical approach to determine the regression line, the method previously used to obtain the value of the unknown is incorrect, giving a line adjusted to $[K^+]$ instead of to PMI, which could lead to serious errors in estimating PMI, and the estimation of the confidence interval is not correct on this formulation. We have made an inverse prediction by changing the variables, which is the recommended approach to solving this problem, and in this way we obtain a line that is now adjusted to the PMI (Figs. 1 and 2). This aspect was previously noted (6,14), and mathematically corroborated by Lang et al. (17). Nevertheless, no alternative formula for calculating PMI was given and, to our knowledge, ours is the only correctly computed equation to date.

In our series, the best results were obtained in cases of rapid death and cases with no metabolic disorders. This might possibly be explained by the absence of in vivo disturbances in the potassium concentration. Most forensic cases in which the estimation of the PMI is required deal with individuals found dead, i.e., nonhospital cases. In fact, the equation to be used is that obtained when only nonhospital deaths are considered: $PMI = 3.92[K^+] - 19.04$. It is worth mentioning that the obtained value of R is 0.83, contrasting with 0.76 when all cases are considered.

To illustrate the differences between our approach and the traditional method, we applied both approaches to our data (Fig. 3). The traditional formula for calculating PMI would be $PMI = 5.58[K^+] - 31.29$ and the proposed formula is $PMI = 3.92[K^+] - 19.04$. The differences between these formulae are obvious. The R^2 value for our estimation is 0.70, contrasting with a lower value for the formula obtained by the "traditional" way of 0.57. This means that our equation is able to explain 70.1% of the variation observed in the data, for only 57.5% using the other one, which would imply an additional error of 29.65%. The magnitude of the error could be better understood considering two supposed values of vitreous potassium. In a case with a $[K^+] = 12$ mmol/L, the estimated PMI according to the conventional formula is 35.67 h, but using our calculation, it is 28. In a case with $[K^+] = 6$ mmol/L, the estimated PMI with the conventional formula would be 2.19 h, but using our calculation this becomes 4.48 h. Moreover, the confidence intervals obtained with conventional formulae are also incorrect as the standard deviation refers to $[K^+]$ instead of PMI, precluding the construction of valid confidence intervals. The exclusion of cases with urea < 30 mg/dL and creatine $\geq$ 0,5 mg/dL further improves the precision of the equation for hospital cases. Different tests were made for several values of both metabolites and only the best results are shown (Table 4). Similar limitations were previously considered by Coe (4) and Madea (7,12). Other factors such as race, age, sampling, technique, and general lack of standardization could explain some of the other differences found.

TABLE 3—*Data provided for the study.*

CASES	PMI †	GROUP	$[K^+]$ ‡	CASES	PMI	GROUP	$[K^+]$	CASES	PMI	GROUP	$[K^+]$
1	1	2	5.5	56	5.66	1	5.7	111*	15.38	1	8.6/8.5
2	1.08	1	5.6	57	5.66	1	6.3	112	15.51	1	8.2
3	1.5	1	5.8	58	5.75	1	6.4	113	15.75	2	12.4
4	1.83	1	5.7	59	5.91	1	6.3	114*	15.83	1	8.6/8.6
5	1.91	1	8.4	60*	5.95	1	6.8/7	115	16.16	1	9.1
6	2	1	6	61	6	2	11.3	116*	16.25	2	9.2/10.1
7	2.08	1	6.3	62	6	1	8.9	117	16.41	1	7.3
8	2.16	1	5.9	63	6	1	6.4	118	16.5	1	8.3
9	2.16	1	6	64*	6.16	1	7/6.5	119	16.66	1	10.9
10	2.25	1	7.3	65	6.25	1	5.7	120	16.83	1	9.7
11	2.41	1	6.2	66	6.33	1	7.2	121*	16.88	1	10.1/10
12*	2.5	1	5.3/5.3	67	6.5	1	8.2	122*	16.91	1	8/8.5
13	2.5	1	6	68*	6.75	1	7.6/7.5	123*	17	1	8.7/9.7
14	2.5	1	6.4	69*	7	2	8.9/9.8	124	17	1	8.2
15	2.58	1	5.1	70	7	1	6.2	125	17	1	9.3
16	2.66	1	5.9	71	7	1	6.5	126*	17.13	1	8.7/8
17	2.75	1	6	72	7	1	6.9	127	17.5	1	8.1
18	2.75	1	5.4	73	7.08	1	6	128	17.58	1	8
19	2.83	1	6.1	74	7.41	2	7.4	129	17.75	1	10.5
20	2.83	1	6.2	75	7.5	1	6.9	130	17.76	1	9.9
21	2.83	1	5.9	76	7.5	1	6.7	131*	17.91	2	8/8.7
22	2.91	1	5.8	77	7.5	1	6.9	132	18	1	9.5
23	3	1	6.1	78*	7.83	1	7.6/6.6	133	18	1	10.5
24	3	1	5.3	79	8	1	7.2	134	18.69	1	9.1
25	3.08	1	5.7	80	8.25	1	6.8	135	18.75	1	8
26	3.08	1	5.6	81	8.33	1	7.4	136*	18.91	2	10/9.8
27	3.25	1	6.5	82	8.5	1	7.6	137	19.08	2	8.3
28	3.33	1	6.8	83	8.5	1	7.3	138*	19.41	1	10.3/9.7
29	3.5	1	5.4	84	8.75	-	9.4	139	19.48	2	14.3
30	3.5	1	6.2	85*	9	2	7.2/7.1	140	19.5	1	8.6
31	3.5	1	5.9	86	9.08	1	6.6	141	19.5	1	9.7
32	3.66	1	6.6	87	9.25	1	7.6	142*	19.58	2	11.8/11.4
33*	3.7	1	6/6.5	88	9.5	2	8.8	143	20	1	8
34	3.75	1	7.3	89	10.25	2	8.7	144	20	2	14.8
35	3.83	1	6.3	90	10.33	1	7.1	145	20	2	10.8
36*	3.86	1	6.5/6.8	91	10.5	2	8.6	146*	20.25	1	8.4/8.9
37	3.93	1	6.3	92*	10.83	2	7.1/7	147*	20.75	1	8/8.2
38	4	1	6.1	93	10.9	1	8.1	148	21	2	9.5
39	4.05	1	5.9	94	11.16	1	8	149	21.75	1	8
40	4.08	1	5.8	95*	11.41	1	7.8/7.9	150	22.25	1	7.6
41	4.25	1	6.1	96	12.25	1	7.7	151	22.25	1	9.7
42	4.5	1	5.9	97	12.5	1	7	152*	22.5	2	12/11.2
43	4.5	1	5.7	98	12.91	2	7.3	153	22.95	1	9.9
44	4.5	1	5.9	99	13	1	10.2	154*	23.5	1	10.8/10.1
45*	4.66	1	6/6.1	100	13.25	1	8	155	24	1	10
46	4.75	1	4.4	101	13.25	1	6.8	156*	24.25	1	10/9.4
47	4.75	1	5.7	102*	13.5	2	7.8/7.8	157	25.25	2	16
48	5	2	5.3	103*	13.75	1	9.9/9.2	158*	25.5	2	12.3/13.3
49	5	1	6.7	104	14	1	8.5	159*	25.5	2	14.5/14.5
50*	5	1	6.5/6.3	105	14.45	1	7.6	160	27.25	2	6.6
51	5.08	1	7	106	14.5	1	7.8	161	28	1	9
52*	5.16	2	8.8/8.5	107	14.68	1	6.8	162	28.91	1	11
53	5.25	1	7.5	108	15	1	8.5	163*	29.66	2	8.5/9.1
54	5.25	1	7.2	109	15.25	1	7.7	164*	40.45	2	22.3/24.1
55	5.5	1	6.6	110*	15.33	1	8.9/9				

* $[K^+]$ determined in both eyes. † PMI in hours. ‡ $[K^+]$ in mmol/L

TABLE 4—*The regression line and the formula proposed.*

	Groups	*N*	Regression Line*	Formula Proposed	*p*	*R*†
All Cases	No levels	164	$[K^+] = 5.358 + 0.229$ PMI	PMI = $2.580[K^+] - 9.307$	<0.001	0.768
	UREA ≥30 mg/dL	125	$[K^+] = 5.107 + 0.256$ PMI	PMI = $2.576[K^+] - 9.314$	<0.001	0.813
	Creatinine <0.5 mg/dL	82	$[K^+] = 5.702 + 0.181$ PMI	PMI = $3.642[K^+] - 17.746$	<0.001	0.813
	Urea ≥30 mg/dL and Creatinine <0.5 mg/dL	52	$[K^+] = 5.485 + 0.217$ PMI	PMI = $3.612[K^+] - 18.033$	<0.001	0.886
Group A‡	No levels	133	$[K^+] = 5.601 + 0.179$ PMI	PMI = $3.923[K^+] - 19.044$	<0.001	0.838
	Urea ≥30 mg/dL	99	$[K^+] = 5.574 + 0.179$ PMI	PMI = $4.086[K^+] - 20.038$	<0.001	0.855
	Creatinine <0.5 mg/dL	79	$[K^+] = 5.592 + 0.199$ PMI	PMI = $3.513[K^+] - 17.007$	<0.001	0.835
	Urea ≥30 mg/dL and Creatine <0.5 mg/dL	51	$[K^+] = 5.495 + 0.217$ PMI	PMI = $3.599[K^+] - 17.923$	<0.001	0.883
Group B‡	No levels	30	$[K^+] = 5.522 + 0.281$ PMI	PMI = $1.670[K^+] - 0.516$	<0.001	0.685
	Urea ≥30 mg/dL	25	$[K^+] = 4.232 + 0.385$ PMI	PMI = $1.952[K^+] - 4.282$	<0.001	0.867

* PMI = hours and $[K^+]$ = mmol/L.

† *R* = correlation coefficient.

‡ Group A: cases without known metabolic disturbance (rapid deaths, including sudden natural death, traumatic death). Group B: cases with some metabolic disturbance (protracted death, death involving hospitalization in Intensive Care Units and cases of natural death from chronic illness).

TABLE 5—*Confidence intervals (95%) for different* $[K^+]$*. Results in hours.*

	All Cases			
$[K^+]$*	No levels	Urea ≥ 30 mg/dL	Creatinine <0.5 mg/dL	Urea ≥ 30 mg/dL and creatinine <0.5 mg/dL
8	11.332 ± 0.766656	11.294 ± 0.812394	11.386 ± 0.929214	10.864 ± 0.89298
12	21.651 ± 1.578456	21.597 ± 1.545588	25.952 ± 2.834766	25.312 ± 2.619936
16	31.970 ± 2.825064	31.901 ± 2.755764	40.519 ± 5.088402	39.761 ± 4.67676
20	42.289 ± 4.129686	42.205 ± 4.03448	55.085 ± 7.377678	54.209 ± 6769422
24	52.608 ± 5.45094	52.508 ± 5.332536	69.651 ± 9.677448	68.658 ± 8.87238
	Group A			
$[K^+]$*	No levels	Urea ≥ 30 mg/dL	Creatinine < 0.5 mg/dL	Urea ≥ 30 mg/dL and creatinine < 0.5 mg/dL
8	12.342 ± 0.714186	12.647 ± 0.812394	11.101 ± 0.840906	10.871 ± 0.90288
12	28.035 ± 2.158002	28.989 ± 2.420154	25.155 ± 2.557764	25.269 ± 2.661318
16	43.728 ± 3.882582	45.331 ± 4.361148	39.209 ± 4.593402	39.666 ± 4.765464
20	59.421 ± 5.635872	61.674 ± 6.336198	53.263 ± 6.66171	54.063 ± 6.90624
24	75.114 ± 7.397478	78.016 ± 8.32095	67.317 ± 8.739522	68.461 ± 9.057708
	Group B			
$[K^+]$*	No levels	Urea ≥ 30 mg/dL		
8	12.842 ± 2.745864	11.336 ± 2.058408		
12	19.521 ± 2.658744	19.145 ± 1.877436		
16	26.200 ± 4.552218	26.954 ± 3.1086		
20	32.880 ± 6.964056	34.762 ± 4.759524		
24	39.559 ± 9.507762	42.571 ± 6.518952		

*[*K*+] in mmol/L.

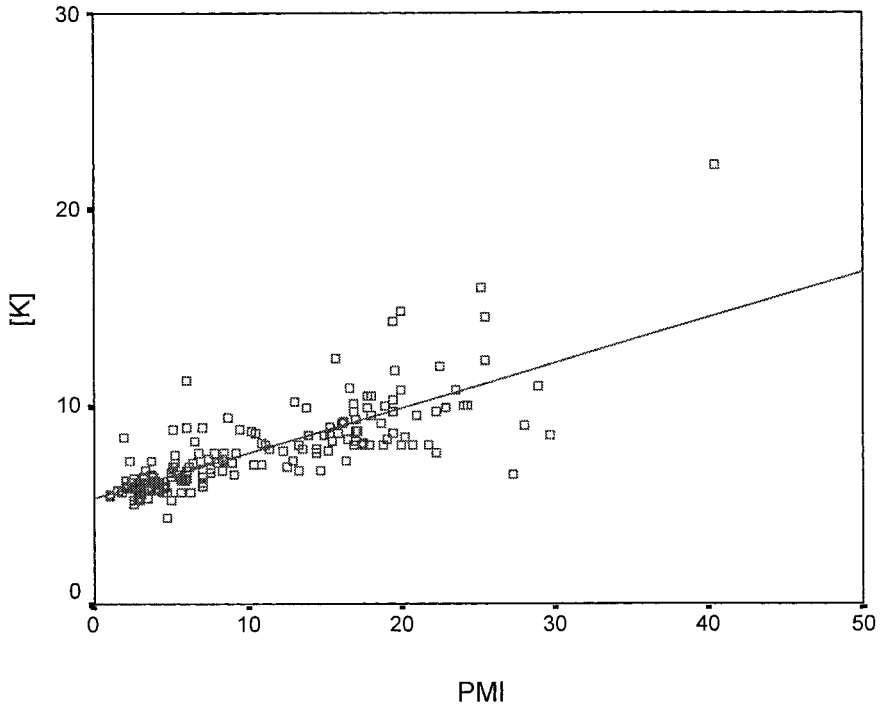

FIG. 1—*Regression line using PMI as independent variable. All data included. PMI in hours and [K^+] in mmol/L.*

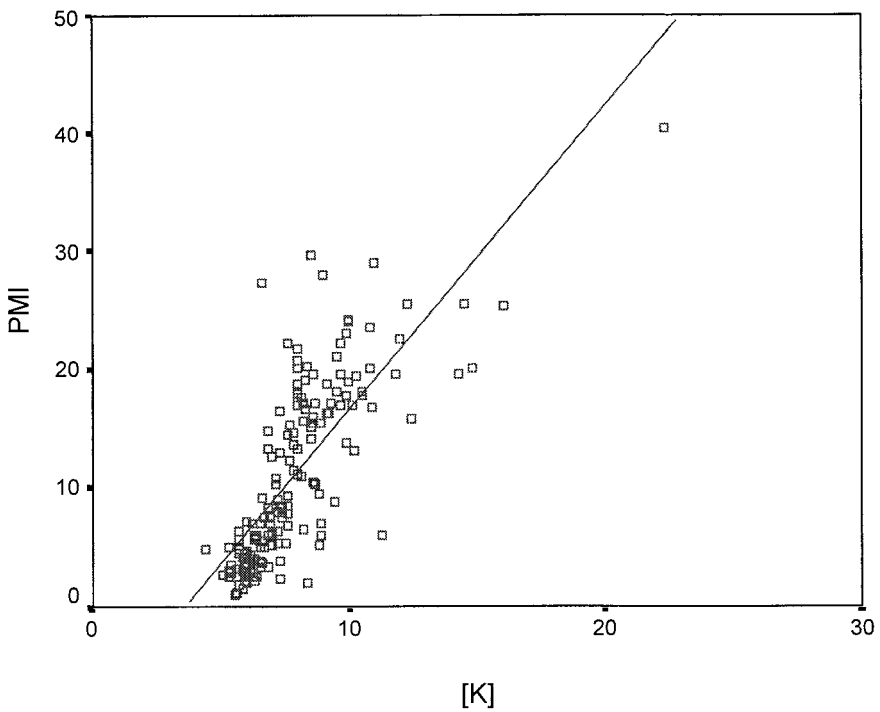

FIG. 2—*Regression line using [K^+] as independent variable. All data included. PMI in hours and [K^+] in mmol/L.*

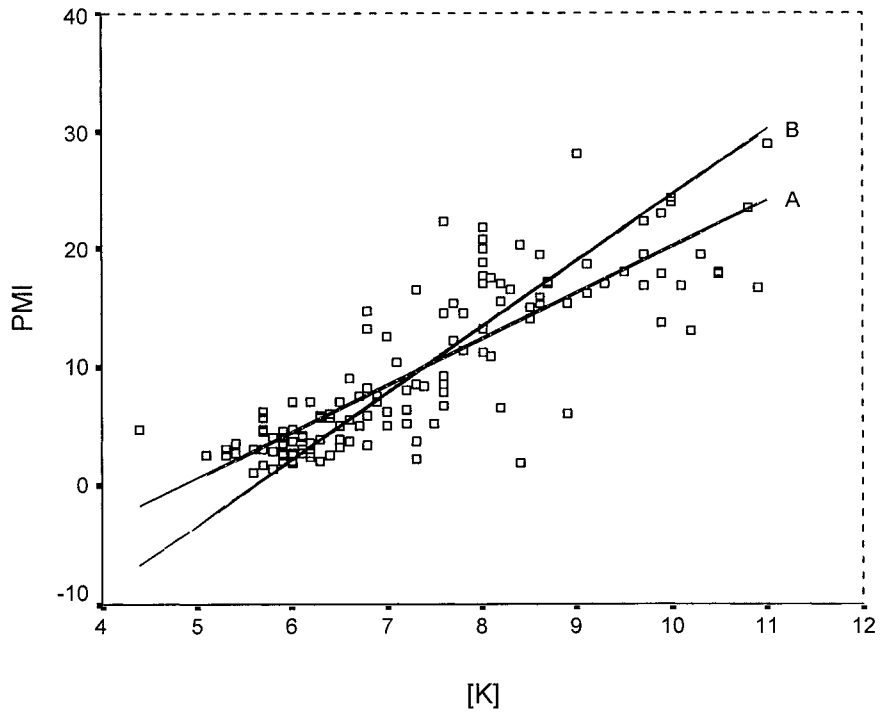

FIG. 3—*The two possible ways to obtain a formula for PMI from vitreous potassium are shown (line* A*: our approach, line* B*: "traditional" approach). Although estimations are similar with potassium concentrations around 7.5 mmol/L, the divergence readily increases from this point in both directions. PMI in hours and [K^+] in mmol/L.*

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