P A R T B

(CHAPTERS III, IV)

P A R T B

CHAPTER III

(Present Investigation)

[Background: the present work had its foundation laid in author's previous published work[65] (discussed earlier in PART-A of this thesis). In that work, a special aeration technic was developed for investigational purposes in such a manner and with such scientific judgement of things prevailing in the circumstances as have helped solve, ideally, the dual original problem of prevention of error from the dual source, intrinsic(volatile metabolites pre--sent normally in test blood) and extrinsic(interfering factors that originate in laboratory air and reagents). The principles and practice adopted in that work were as reviewed below: with the aid of a two-armed(two-winged) apparatus, a two-pronged attack was hurled on the dual source of error, intrinsic and extrinsic, with a view to fight out the vitiating factors in a dual manner. Paired things and paired events were thus a surprising coinci--dence in that work. The sequence of events were as follows: (i) vicious laboratory air was drawn in through the apparatus, designed and constructed in two wings in such a manner and with such careful details as to ensure

to ensure absolute parity in its two wings, one designated as the test bleed wing charged with test bleed and reagents, and the other designated as the control wing charged only with the same quantity of corresponding reagents taken out from common reagent bottles; (ii) the two wings were subjected to aeration experiment, conducted under identical physical conditions and for the common duration. It follows therefore that, under identical physical conditions of aeration experiment conducted on both wings of apparatus for the common experimental duration, identical volumes of vicious laboratory air and therefore, identical quanta of vitiating factors (present in identical volumes of air from common laboratory source) were drawn in through both wings of apparatus (vide Avogadro's Law). The quantum of error from laboratory air as an extrinsic source, thus included in each wing, was represented by a value (say, Va). Similarly, identical quanta of volatile factors released from identical quantities of reagents (taken up in both wings), were represented by value (say, Vr). The total quantum of error (from laboratory air and reagents) thus injected into either wing, was therefore represented by common value (Va+Vr=say, Ve).

Therefore, after the intrinsic vitiating metabolites (of value Vi) were directly eliminated, en masse, in the wash-trap (B) of .....

of apparatus (Fig.1, reprint interposed between pages 72 and 73), the iodometrically determined value in the two experiments, test and control, was as noted below:

Experiment on test blood in test wing:	Experiment on control sample in control wing:
Value = Vt+ [Ve] (=Va+Vr)	Value = [Ve] (=Va+Vr)

The value [Ve] was thus a common error in the two experiments. The calculated difference in the two values, (Vt + [Ve]) and [Ve], represented, therefore, the absolute value (Vt) for true alcohol content of the test blood so arrived at.

The quantitative alcohol-error vicious relation (Vt + [Ve])** that continued to be a permanent feature in previous methods (vide expression(2), page 38) was thus broken in the work and a new order of law-science relationship established at its ideal level of perfection and security in the matter of detection of the guilty (of drunkenness) and protection of the innocent. But, with all that has been said, suggested and implemented in the matter of arriving at the absolute value (Vt) for true alcohol, an element of presumption (without verification of its correctness) was made in the said work: because aeration experiment was conducted in the two identical

alcohol-error vicious relation broken and a new order of law-science relationship established at ideal level of perfection

**representative terms Vt, Vi and [Ve] were explained in in page 36 and their relative significance indicated in algebraic expressions (1,2,3,4), pages 37-40

identical wings of apparatus, subjected to identical physical conditions and experimental time-lag, it was presumed (vide Avogadro's Law), that identical quanta of error (of value [Ve]) was injected into and oxidised by standard potassium dichromate set up in either wing of apparatus. It was on the basis of this presumption that error [Ve] was eliminated as a common factor (vide 'scheme' supra). The presumption thus made has, however, raised a point of doubt in the author's mental horizon that urged him to investigate further into the dual original problem on a revised program that has left no room for doubt and thus paid the highest dividend in results embodied in this PART-B of the thesis.

1. INVESTIGATION ON REVISED PLAN

In the revised plan of investigation pure air was proposed to be employed for the purpose of vacuum diffusion-separation of alcohol from the test blood. In this project, the extrinsic vitiating factors present in laboratory air were proposed to be blocked by bubbling of vicious laboratory air through a wash-trap provided in the apparatus (detailed later). Therefore, the element of doubt since harboured in the author's

author's mind on the question of distribution of identical volumes of air and therefore, identical quanta of vitiating factors(from laboratory source) in the two wings of apparatus,employed in the previ--ous work(vide 'background',pages 80-83)was proposed to be removed in the present work. Pure air thus released was employed in regulating the kinetics in--volved in the process of separation (a)of alcohol from test blood, fractionation (b) of the released alcohol from the intrinsic normal volatile metabo--lites (simultaneously released) and its transport (c) for final absorption-oxidation in standard pota--ssium dichromate-sulphuric acid mixture set up in the apparatus (detailed later).

1.1 Theoretical Approach

Solution to problem of prevention of error in blood alcohol test results involved solution to two problems(as discussed previously in PART-A):(i)prob--lem posed by intrinsic normal volatile metabolites (normally present in test blood); (ii)problem posed

posed by interfering factors likely to be present in laboratory air and reagents as extrinsic sources of error. For clarity in understanding, the sequence of events that figured in the present work, it was thought convenient to start discussion at the stage where no precaution was included in the technic to exclude error (in results) from either of the dual source, intrinsic and extrinsic (i, ii), and then proc-eed with the technical details of the program proposed to be completed with a view to win over these sources of interference. For this purpose, expression (1), PART-A, page 37, was selected as the model of reference and is reproduced below:

Vg	=	Vt	+	Vi	+	Ve (=Va+Vr)
(gross value determined iodometri-cally)		(value for true alcohol)		(value for normal intrinsic volatile metabo-lites)		(value repre-senting the sum total of two values, Va for extri-sic factors prevailing in laborato-ry air and Vr for facto-rs present in reagents)

Author's assignment in the program was to solve problem of prevention of error of value Vi + $\boxed{Ve}$ (=Va+Vr) thus included in results as noted in the above expression and arrive at the highest level of accuracy as defined in expression(4), Vg=Vt, PART-A, page 40. This level of accuracy was proposed to be attained by successive elimination of error from the dual source of interference(discussed below in chronological order).

Prima facie, because pure air was proposed to be employed in the present work, error(of value Va) from laboratory air as an extrinsic source was directly prevented and therefore, the position represented by above expression, Vg=Vt+Vi+ $\boxed{Ve}$ (=Va+Vr), then assumed as visualised in expression,

$$Vg = Vt + Vi + (\boxed{Ve} - Va = Vr)$$

OR

$$Vg = Vt + Vi + Vr \quad \ldots\ldots\ldots \quad (e)$$

(Va having been eliminated)

Error of value(Vi) from intrinsic volatile metabo--lites and of value(Vr) from reagents(as the other

the other extrinsic source of interference) was proposed to be prevented in two stages as follows:
(i) by fractionation of the released alcohol from intrinsic volatile metabolites (freed simultaneously). Error of value (Vi) for intrinsic volatile metabolites was thus visualised as prevented and the position then assumed as noted in the next stage,

$$Vg = Vt + Vr \quad \ldots\ldots\ldots (f)$$

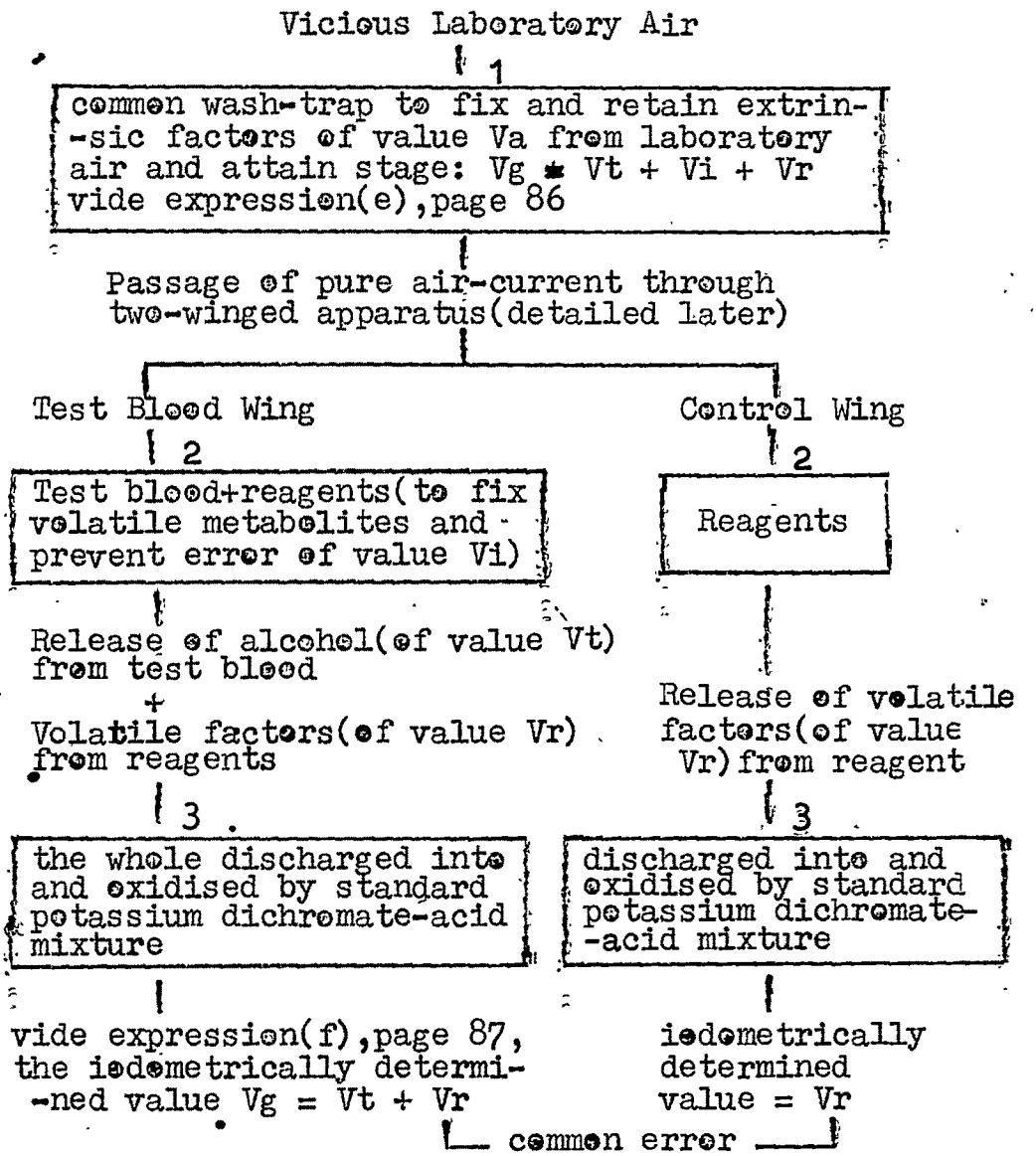
(ii) by control test as the indirect method proposed to be adopted in eliminating error (Vr) from volatile factors likely to be present in reagents as the other extrinsic source, when the final position would have assumed as the position of absolute value (Vt) for true alcohol as visualised in the expression,

$$Vg = Vt \quad \ldots\ldots\ldots\ldots (g),$$

where the term Vg ceased, obviously, to be designated anymore as the gross value and assumed as the real value (Vt).

The processes (a, b, c-page 84) of separation of alcohol, its fractionation from intrinsic volatile metabolites and transport for absorption-oxidation in standard acid dichromate set up in the apparatus (detailed

(detailed later)were as summed up in the diagram (below)along with successive results(e,f,g)aimed at:



Error of value(Vr)was proposed to be thus eliminated as a common factors by calculating the difference of

difference of two values, (Vt+Vr) and (Vr), iodometri-
-cally determined in the two experiments, conducted
on test blood in one wing and on the control sample
(reagents) in the other wing. The difference (Vt) of
the two values represented, therefore, the absolute
value for true alcohol content of test blood thus
arrived at as reality, represented in the expression,

$$Vg = Vt \quad \ldots\ldots\ldots (g),$$

where the term Vg ceased, obviously, to be designated
as gross value and assumed as the real value (Vt).

2. EXPERIMENTAL DETAILS

The theoretical principles, enunciated previously
in pages 86,87 to fight out the vitiating factors
present in laboratory air (as an extrinsic source) in
particular and the dual source of error in general,
were experimentally translated into practice in the
present work. The five imaginary boxes (1), (2,2) and
(3,3), shown in the model diagram, page 88, assumed
tangible shape of five corresponding glass flasks

glass flasks(having corresponding functions)as com--ponents of the assembled two-winged apparatus(des--cribed later) that was employed in the present investigation.

2.1 Method

A special vacuum diffusion-oxidation micro method was required to be developed for the purpose. As indicated previously in the model diagram,page 88, the vicious laboratory air was first purified by bubbling through a wash-trap set up in the apparatus. Pure air thus released was employed in creating vacuum in the test blood container in one wing and in control sample container in the other wing. Further details were as explained fully in the figure (blue print interposed between pages 91 and 92).

2.2 Reagents

1.Scott-Wilson reagent(vide J.Biol.Chem.16:281,289, 1914) for fixing and retaining the normal volatile metabolites present in test blood; 2.Potassium dichromate,0.1 N; 3.Sodium thiosulphate,0.05N and 0.01 N, to fill in two 2-ml micro-burettes, each

each capable of being read to 0.01 ml; 4.Potassiun iodide crystals,reagent grade; 5.Concentrated sulphuric acid,reagent grade; 6. 1% Starch solution freshly made every day.

2.3 Apparatus

The design and construction of apparatus in its two wings and orientation,function etc of its components are fully explained in figure in blue print interposed between this page and the next page 92.

2.4 Procedure

Test blood: residual blood(received at this laboratory for alcohol test by author's previous method vide J. & Proc.Inst.Chem.,Vol.XXXIV,Pp.193-196,July, 1962)was selected in the present work.Varying known quantities of standard alcohol(vide 'infra') were added to measured volumes of test blood and experimentally recovered.

Standard alcohol: two standards,one with 1 in 500 dilution and the other with 1 in 2000 dilution,were prepared from absolute alcohol. 1 ml of each was

each was directly oxidised with 0.1 N potassium dichro-
-mate-concentrated sulphuric acid mixture(each of 10 ml) and their alcohol values,iodometrically determined,were respecively 0.9773 mg and 0.2443 mg.

Into the wash-trap(flask A,figure in blue print)and the reaction flasks(C,C)were measured out the reagents(FM)as noted in the figure. 0.10 ml Scott-Wilson reagent was pipetted into the test glass cup(B),followed by 0.10 ml test blood run from Ostwald-Folin pipette,rinsed out with 0.1 ml of glass-distilled water that was added to test blood in cup(B).The cup was then rolled between the palms to mix the contents(BRt,fig.1).A known quantity of alcohol was then added to it and mixed.Into the control cup(B)was pipetted 0.10 ml Scott-Wilson reagent(Rt)alone.The cups (B,B)were then connected as shown in the figure,the aspi-
-rator pump set to work and the air-current* continued for 15 minutes at the temperature of boiling water-bath.

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* It should be noted that the lower openings of the inlet capillary glass tubes (ict, figure in blue print) are clear 5 mm above the surface of liquid contents of glass cups(B,B). The air-current, harnessed in the technic,was not obviously intended for bubbling of air through these liquids(as is followed in conventional aeration technic), but for creating vacuum only within these vessels.

During that interval, the contents of cups(B,B) dried up and all volatile substances(thus released) also absorbed in standard acid dichromate in reaction flasks(C,C) and oxidised.

Kinetics involved in the process: pure air was rel--eased after bubbling of air-current through standard dichromate-sulphuric acid in flask(A), which functioned as wash-trap to fix and retain the volatile contamina--nts present in laboratory air. Subsequent events in the process of diffusion-separation of alcohol from test blood and transport of the released alcohol(and volatile matter present, if any, in reagent mixed with blood) for its absorption-oxidation by standard pota--ssium dichromate(RM) in test and control reaction flasks(C,C) were regulated by kinetics involved in the technic: (i) disparity in the calibre of inlet capilla--ry glass tube(ict of 0.2 mm diameter) and outlet tube (OT of 4.0 mm diameter) ensured disparity in the larger volume of air exhausted per unit of time from glass cups(B,B) than the volume that entered into these vess--els. The resultant effect was creation of vacuum and

and rapid diffusion of alcohol from the test blood in test cup(B)and other volatile substances prese--nt,if any,in the reagents employed in fixing the intrinsic volatile metabolites.Similar set of even--ts occured also in the control glass cup(B) with similar release of volatile factors present in the reagents,similarly absorbed and oxidised by stand--ard dichromate set up in either wing of apparat--us(figure in blue print); (ii)in its passage through narrow inlet capillary glass tube in cups (B,B),the air-current* had to force its way in against appreciable resistance offered by the former,and this force(pressure)was exhibited in sharp jets striking and agitating the liquid con--tents.This agitation also helped accelerate sep--aration of volatile matter in test and control samples; (iii)high vapour pressure of alcohol (about 10 times that of aqua)was another contri--butory factor in the separation process;

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*The pure air-current was harnessed in the proce--ss to perform a dual role: creation of vacuum in glass cups(B,B)and as a mechanical carrier for transport of volatile substances to reaction fla--sks(C,C).Please also refer to footnote,page 92.

(iv) distillation of the specimen under reduced pressure was also an accompaniment; (v) temperature of boiling water-bath generally helped accelerate processes(i,ii,iii,iv); (vi) the cargo of diffused components that filled in the air spaces in cups(B,B) were then carried by the air-current for its final discharge into and oxidation by corresponding reaction mixture in flasks(C,C), as shown in figure in blue print.

A series of experiments was conducted with addition of different known quantities of standard alcohol to 0.1 ml of test blood. The calculated difference in volume of dichromate reduced in control and test reaction flasks(C,C) (figure in blue print), determined iodometrically, represented the equivalence of true alcohol thus recovered (1 ml of 0.1 N potassium dichromate reduced is equivalent to 1.15 mg of alcohol oxidised) and noted against the total of original alcohol content plus the quantity added(vide results of recovery, table 1 below).

2.5 Results of recovery: Table 1

I Expt.	II Test blood in ml	III Original alcohol content in mg	IV Added alcohol in mg	V Total alcohol (III+IV) in mg	VI Total alcohol recovery** in mg	VII % rise(+) or fall(-) in recovery
1	0.10	0.0305	0.0517	0.0822	0.0819	- 0.36
2	0.10	0.0305	0.1035	0.1340	0.1347	+ 0.52
3	0.10	0.0305	0.1552	0.1857	0.1864	+ 0.48
4	0.10	0.0305	0.2070	0.2375	0.2364	- 0.46
5	0.10	0.0305	0.2587	0.2892	0.2881	- 0.34
6	0.10	0.0305	0.3204	0.3509	0.3494	- 0.42
7	0.10	0.0305	0.4239	0.4544	0.4556	+ 0.26
8	0.10	0.0305	0.4756	0.5061	0.5085	+ 0.47

** Figure represented mean of duplicate test.

Corresponding reduction of dichromate in wash-trap (flask A, figure in blue print) of all experiments (1 to 8, Table 1) was as drawn up below:

Table 2

Reduction of dichromate***in was-trap (flask A, figure in blue print between pages 91 and 92)

I Experiment	II Vol. of 0.1 N $K_2Cr_2O_7$ reduced	III Alcohol equivalence in mg (figure in II multiplied by 1.15)
1	0.0020	0.0023
2	0.0030	0.0034
3	0.0020	0.0023
4	0.0010	0.0011
5	0.0020	0.0023
6	0.0020	0.0023
7	0.0010	0.0011
8	0.0030	0.0034

***Quantum of error likely to be included from extrinsic volatile factors present in laboratory air would depend, obviously, on the technic employed (diffusion or aeration or distillation) and on time-lag, during which the experimental procedure was exposed to laboratory atmosphere.

Fluctuation in figure of reduction of dichromate is a pointer to changing condition of laboratory atmosphere during the day's business.

3. SUMMARY AND DISCUSSION

The present work has helped remove doubt(harboured in author's mind) as pointed out in 'background'pages 80-83 and thus built up the superstructure on the foundation laid in the previous work[66] (discussed in PART-A,chapter II): because the vicious laboratory air was filtered through acid-dichromate as wash-trap (flask A, figure in blue print) that retained extrinsic factors from this source, the uncertainty since prevailing over entry of equal or unequal volumes of pure air (thus released) through the two wings of apparatus(figure in blue print between pages 91 and 92) did not,obviously, figure at all or matter anything in the present work. There would be also noticed a surprising coincidence of paired things and paired events displayed in the present inves--tigation,conducted with a view to solve a paired prob--lem:with the aid of a two-armed(two-winged)apparatus(vide figure in blue print),the two-pronged attack(experiments on test and control samples),hurled on the dual problem of

problem of prevention of error from the dual source, intrinsic and extrinsic, has ensured total success as contemplated. The plan, the material and the method of investigation were integrated in such a manner and with such scientific judgement of things prevailing in the circumstances as have moved and acted in the right direction to pay the highest dividend in test results: (i) the intrin--sic normal volatile metabolites (ketone bodies etc) were fixed and eliminated by Scott-Wilson re--agent$ that retained them (vide 'procedure' supra); (ii) the extrinsic contaminants were pre--vented in two instalments- (a) volatile contami--nating factors present in laboratory air were fixed and retained in the process of bubbling of air-current through the mixture of standard pota--ssium dichromate-sulphuric acid in wash-trap (flask A); (b) volatile contaminants present in reagents as the other extrinsic source were indi--rectly eliminated by control test conducted in the control wing of the apparatus (figure in blue

blue print between pages 91 and 92). Inter alia, the control test so arranged has also served as an excellent 'blank' experiment, automatically conduc--ted on volatile substances present, if any, in the chemicals, laboratory distilled water etc employed in the work for iodometric determination of alcohol in the test blood. The two-winged apparatus, designed and constructed on original lines for purposes of this original investigation, was forced as it were to perform the key role in integrating the vital processes(i, ii-a, b) that ensured total success in implementing the author's assigned program to fight out the dual source of vitiating factors and thus solve the original dual problem as scientific rea--lity, secret of which was not unfolded previously. Thus, the two LINES OF DEFENSE(drawn up previously in pages 22 and 43) against vitiating factors from the dual source, intrinsic and extrinsic, were effi--ciently maintained and accuracy in test results attained, therefore, at its highest level(humanly possible to attain) as defined in expressions(3)., page 40 and (g), page 89: $V_g = V_t$.

Experimental recovery of alcohol from the test speci-mens (Table 1, page 96) was in excellent agreement with the quantity added in each case: accuracy in resu-lts was ensured at 100 % ± 0.4 accurate (average of 8 experiments thus conducted).

Reduction of standard dichromate (added to sulphuric acid in flask A vide blue print between pages 91 and 92), determined iodometrically and drawn up in Table 2, served as index to presence of contaminants in labora-tory air and their quantum as carried with the air-current (and discharged into and oxidised in the wash-trap) during the operation of aeration.

The results thus attained and embodied in the thesis, have left, therefore, no room for presumption or doubt to crop up (vide 'background', pages 80-83), no room for challenge from the judiciary or legal profession (as referred to previously in section I-d of 'introduc-tion', chapter I, pages 29-32), no room for adverse public criticism on the point of accuracy in the deter-mined figure of bio-chemical index of drunkenness(i.e. blood alcohol concentration in alcoholic intoxication). Stated in a nut-shell, this investigation has thus

has thus removed the misgiving hitherto prevailing on the point of accuracy (and security) in the determined blood alcohol index that has been ensured as scientific reality in the present work.

A skeleton summary of the work has been submitted recently for publication under the title "LOW VACUUM DIFFUSION-OXIDATION MICRO TEST FOR BLOOD ALCOHOL"*.

*the published literature would be presented at the time of oral/practical examination.