5. No differences have been detected between the hexosephosphoric acids or their salts, whether derived from glucose, fructose, or mannose.

6. On hydrolysis of the acid by boiling, phosphoric acid is set free and fructose formed. No other hexose could be identified, but the solution, after hydrolysis, was always less lævorotatory than a solution of pure fructose of the same reducing power.

7. The salts of lead, barium, silver, and calcium have been prepared.

[The compound containing phosphorus, which was considered to be phenylhydrazine phosphate, has since been examined by von Lebedew ('Biochem. Zeits.,' 1909, vol. 20, p. 113), who regards it as a phenyl hydrazido-phosphoric acid compound of hexosazone. A re-examination of this substance by the author leads to the conclusion that it is in reality a derivative of hexosephosphoric acid, but decisive results as to its constitution have not yet been obtained.—*November* 15, 1909.]

The Comparative Power of Alcohol, Ether, and Chloroform as measured by their Action upon Isolated Muscle.

By Augustus D. Waller, M.D., F.R.S.

(Received and Read June 24, 1909.)

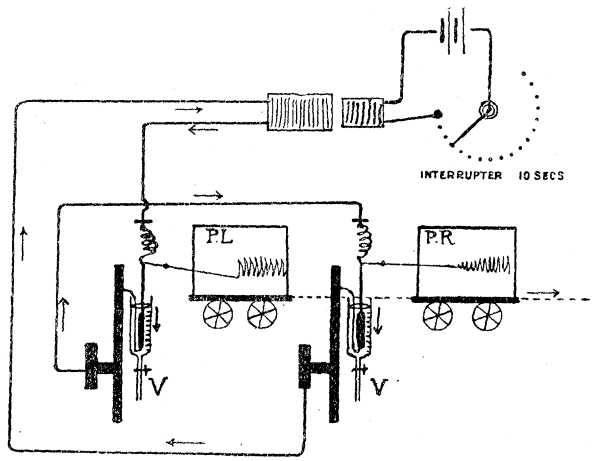
The object of the following communication is twofold: (1) to present the results of a careful comparison of the physiological effectiveness of certain narcotics, and (2) to illustrate the degree of accuracy of which such comparisons are susceptible by the systematic use of the sartorius muscle of the frog as an indicator.

Method.—The two sartorius muscles of a frog are dissected out and the portions of bone to which they are attached are ligatured with fine copper wires serving as conductors. The muscles are set up in the two vessels V, V and connected with two myographic levers that record their movements on two smoked plates L, R. The connections with the secondary coil of an inductorium (Berne model) are as given in the diagram, so that both muscles are traversed in series by the same current in the same direction. The muscles are directly excited once every 10 seconds by maximal break induction shocks. Each observation consists of three parts: a first part consisting of the normal responses of the muscle immersed in normal saline (0·6 per 100 NaCl in tap water); a second part consisting of the responses while the muscle is immersed in an experimental solution; a third part

consisting of the responses while the muscle is replaced in normal saline. The solutions are changed by being run off through a tap and run in from a pipette, care being taken that the volume of fluid is always the same. The induction currents are kept going automatically throughout an experiment, excepting during the short periods required for changing the solution. The apparatus used for this purpose consists of: (1) a Berne coil fed by a 2-volt accumulator; (2) a Brodie clock with interruptions set at six per minute; and (3) a relay key, *i.e.* that shown by G. R. Mines at the July, 1908, meeting of the Physiological Society.

As a general rule of procedure in any comparison between the effects upon two muscles L and R of two solutions A and B, a first comparison is made between the effects of A on L and of B on R, and a second comparison of the effects of B on L and of A on R. Each complete experiment thus comprises two pairs of simultaneous trials of two solutions in reversed order of action, and constitutes an *experimentum crucis* in the strict sense of the term.

Electrical excitation of the muscles while immersed in the experimental solutions—in spite of the fact that the induction currents are in large measure short-circuited by the solution—was systematically adopted in preference to excitation of the muscle after the solutions had been run off, because it affords a more complete picture of the gradual effects of such solutions. Currents of sufficient strength are taken to give maximal excitation in spite of the derivation.



Double Myograph to test Action of Substances in Solution.

The diameter of the muscle vessel was slightly less than 3 cm., so that 30 c.c. of fluid gave in it a column about 5 cm. long, more than sufficient to keep the muscle wholly immersed.

The exciting currents passed through the muscle and solution—principally through the former by reason of the copper wires by which it is attached—are taken of such strength as to give assuredly maximal effects. Their direction is not a matter of indifference, the contractions being always unequal to the two directions of excitation; as a rule, but not always, the more effective direction was from tibial to pelvic end, and this was therefore taken as the ordinary direction of exciting currents. But this is not a very essential point, all that is really necessary is to keep to one direction during experiment. Unpolarisable electrodes are also unnecessary, as, indeed, may be readily seen from the records obtained. The magnification of contraction by the lever was × 2.

(From October 9 onwards I used narrower muscle tubes, in order to use up less fluid for each bath, and to have a greater density of current passing through the immersed muscle.)

Alterations of current distribution caused by alterations of resistance of the experimental fluids; the oligodynamic action attributable to the use of copper wire; small differences of room temperature; the possible excitation of intramuscular nerve as well as of the muscle itself, are the principal circumstances that have been considered and recognised to be negligible in the present connection. On the other hand, every care has been taken to secure constant strength of stimulation and constant pressure of the myographic levers against the recording surfaces, which are moved past the levers in tandem by the same clockwork. The influence of considerable differences of temperature was specially examined (*vide infra*).

By preliminary experiments it was found that conveniently graded effects upon muscular excitability were produced by a 5 per 100 solution of alcohol, by a 1 per 100 solution of ether, and by a 1 per 1000 solution of chloroform (by volume in each case). These strengths are of the order of molecular (5·8 c.c. per 100) in the case of alcohol, decimolecular (1·03 per 100) in that of ether, and centimolecular (0·8 per 1000) in that of chloroform.

Thereafter solutions were made up on a molecular scale, taking as the standard of reference a molecular solution of absolute alcohol, and as the first terms of comparison a decimolecular solution of ether and a centimolecular solution of chloroform as tabulated below.

	Sp. gr.	Mol. wt.	c.c. per 100 c.c. saline to give molecular solutions.
Alcohol	0·79	46	5·8
Ether	0·72	74	10·3
Chloroform	1·50	119·5	7·95

Comparisons were systematically made (1) between alcohol and chloroform; (2) between alcohol and ether; and (3) between ether and chloroform. Such comparisons were, whenever possible, made upon the same muscle, preliminary experiments having shown that two or more successive intoxications, if not too profound, by the same strength of solution, are of equal gravity.

The principal indication of the comparative effects of reagents consists in the rate at which the contractility is abolished in solutions (in 0·6 per cent. NaCl in tap water) of various strengths. The rate and amount of return of contractility in 0·6 per cent. NaCl affords confirmatory evidence, of which, however, we have not made systematic use, having done no more than note the facts: (1) that at equal times of immersion the time required for recovery augments with augmented strength of solution, and (2) that at equal strengths of solution the time required for recovery augments with augmented time of immersion.

Comparisons may be established between: the effects of two solutions upon the same muscle successively; or between the effects of two solutions upon two muscles simultaneously; and each kind of comparison has its own obvious advantage and disadvantage. By the method we have adopted of simultaneously recording the contractions of two muscles in series, we secure the advantage of both plans, and minimise the disadvantage of successive comparison by reversing the solutions on the two muscles. Other obvious advantages of the double method are that we get double the number of observations, and that we can readily tell whether an accidental irregularity is due to the stimulus or to the muscle or to the solution.

We may also compare the effects of different solutions upon different muscles, but in such comparisons from muscle to muscle we must take care that the conditions of observation are, as far as possible, identical. We may not, *e.g.*, compare fresh with stale muscle, nor muscles of greatly unequal bulk, nor muscles taken from healthy and unhealthy frogs, nor results obtained at different temperatures. Nevertheless, comparisons of this order are practically available, for under similar conditions the results of experiment with a given solution are closely similar upon different muscles; the "idiosyncrasies" of different muscles are not a very disturbing factor, although, as might be expected, effects are more rapidly produced with very small than with very large muscles.

The two chief fallacies in their order of importance are: (1) a variation in length of the column of fluid, and (2) a considerable variation of temperature.

As regards the column of fluid, it is evident that this must be kept of constant length during an observation, since the fluid forms a derivation circuit

surrounding the muscle, which is traversed by only a small fraction of current. The effect of varying the length of column is easily shown by adding or taking away fluid while a series of contractions is in progress. I have, therefore, always been careful to replace fluids by pipette as exactly as possible. If, as has sometimes happened, the difference of excitability and contraction in two muscles has been grossly unequal—even more so than in the case of the pair of muscles used for the record of fig. 1—I have thought it permissible to adjust the tubes in their holders upwards or downwards so as to alter the current lines in suitable degree. But once fixed in position, the tubes must not be shifted again; the level of fluid must be kept unaltered throughout experiment.

Differences of resistance between different fluids are in most cases of little moment, *e.g.* a cubic centimetre of chloroform does not increase the resistance of a litre of saline enough to influence the exciting current traversing the muscle. In some cases, however, differences of resistance may be such as to affect the current distribution and the response of the muscle, *e.g.* a 10-per-cent. solution of alcohol in saline has an appreciably higher resistance than saline alone.

It may be objected to the method that excitation is not restricted to the muscular substance, but includes intramuscular nerve tissues. To meet this objection I compared the effects on fully curarised and on uncurarised muscle, and found that they were indistinguishable. This fact, however, is of little weight, inasmuch as immersion in normal saline is of itself sufficient to remove the effect of curarisation. But, on reflection, the objection itself is of little weight. As is well known, the direct excitability of muscle outlasts its indirect excitability *viâ* nerve; loss of all contractility is of necessity loss of direct excitability, and whatever might be said as to the beginning of an observation, there can be no doubt that at its end we are dealing with muscle and muscle only; even at the beginning of an observation, since we are using a strength of current more than sufficient to excite muscle as well as nerve, the contraction must be by direct muscular excitation; and even if it were not, if it were by indirect excitation at the beginning, the comparative results of, *e.g.* the action of ether and chloroform, would remain acquired. To use as a test object excitation of the nerve of a nerve muscle preparation would, of course, be a different method, by which the tissue specially under investigation would be the end-plate. To use as an index the minimal strength of stimulation giving contraction would again be a different method, by which at first indirect and later direct excitability would be investigated. I have avoided both these proceedings, and have preferred to follow the method described because it is more practicable and less ambiguous

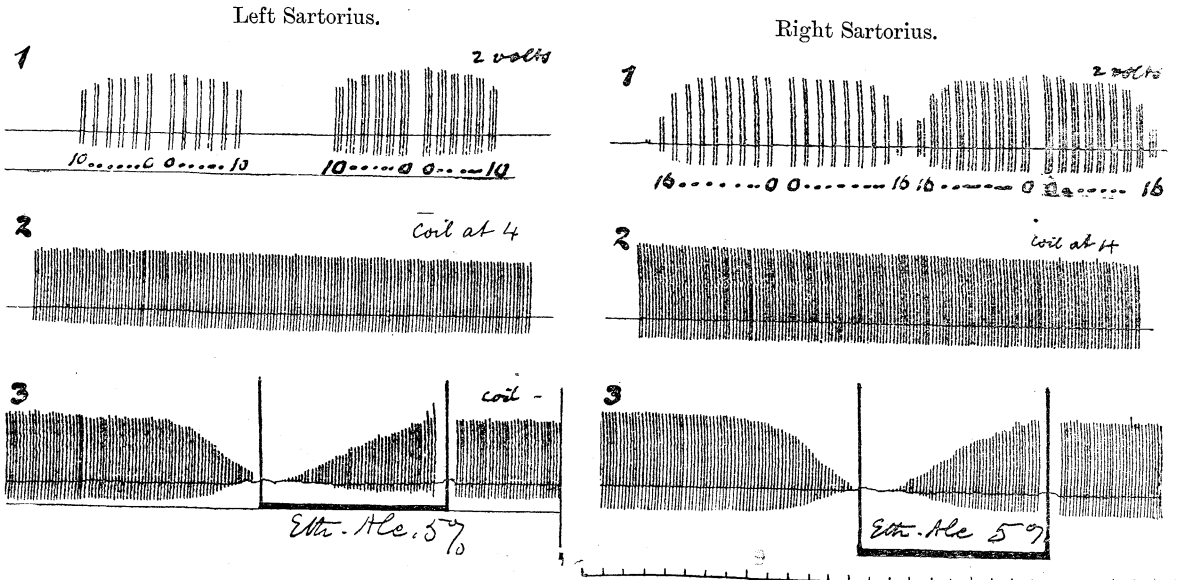


FIG. 1.—September 19, 1908. Temperature = 18°. Simultaneous Records of the Two Sartorius Muscles of a Frog taken on the Double Railway Myograph.

The first pair of records is by groups of contractions with increasing and decreasing strengths of excitation—on the right with the secondary coil at 16, 14, 12...0 ; 0, 2, 4...16 cm. ; on the left at 10, 8, 6...0 ; 0, 2, 4...10 cm.—*i.e.* the apparent excitability happens to be greater on the right than on the left.

The second pair of lines gives the two series of normal contractions by maximal excitations at 10-second intervals with the secondary coil at 4 cm.

The third pair of lines shows the effect on the two muscles of a 5-per-cent. solution of alcohol. The period of immersion is shown by the black lines. The effect of the alcohol on the two sides is equal, although apparently the original condition of excitability is not identical on the two sides ; the illustration has, in point of fact, been chosen for this reason, as it indicates that an identity of excitability need not absolutely be secured. The apparent inequality is principally due to differences of current distribution on the two sides ; it can be corrected by slipping the glass tubes up or down in their holders so that the depth of immersion of the muscle is altered until both muscles respond more nearly alike to a given excitation. The solution in which the muscles are normally immersed is 0·6 per cent. NaCl in tap water.

in its results. I felt justified in making this choice by the results of experiments made many years ago on direct and indirect excitability, and on the junction between nerve and muscle.*

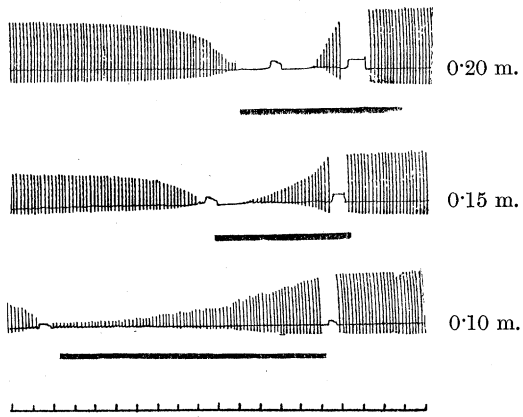


FIG. 2.—August 21, 1908. Effect of Ether Solution at Strengths of 0·1, 0·15 and 0·2 m. (= 1, 1·5 and 2 per cent. by volume).

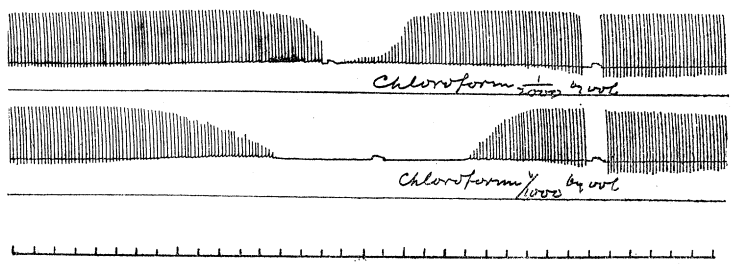
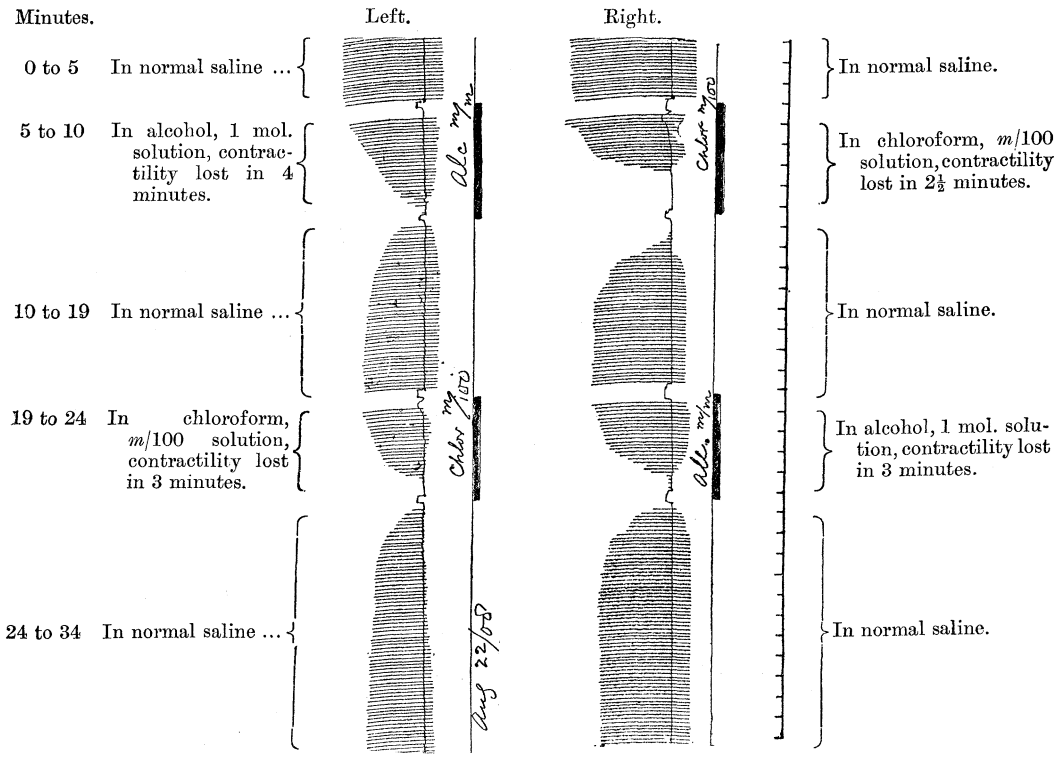


FIG. 3.—August 20, 1908. Comparison between the Effects of Chloroform in 1/1000 and 1/2000 dilution in normal saline (= m/80 and m/160) and of Ether in 1/100 dilution (= m/10).

In chloroform 1/1000 contractility is abolished in 5 minutes.
„ 1/2000 „ „ 12 „

* "Experiments and Observations relating to the Process of Fatigue and Recovery," First Report, 'British Medical Journal,' July, 1885; Second Report, 'British Medical Journal,' July, 1886. In the present connection the principal conclusion of the investigation (carried out during my tenure of a research scholarship to the British Medical Association) was to the effect that the junction between nerve and muscle is functionally a weak link in the neuro-muscular chain, being the first to suffer in its transmitting function in fatigue (by indirect excitation) in intoxications ("curarisation") and in pathological degeneration.

I. *Alcohol* v. *Chloroform.*



(Continuation of record not reproduced.)

35 to 40	In alcohol, 1 mol. solution, contractility lost in 5 minutes.	In chloroform, *m*/100 solution, contractility lost in 5 minutes.
40 to 50	In normal saline	In normal saline.
50 to 55	In chloroform, *m*/100 solution, contractility lost in 4 minutes.	In chloroform, *m*/100 solution, contractility lost in 3½ minutes.
55 to 70	In normal saline	In normal saline.
70 to 73	In ether, 0·2 mol. solution, contractility lost in 1 minute.	In chloroform, 0·02 mol. solution contractility lost in 1½ minutes.
73 to 83	In normal saline	In normal saline.
83 to 88	In alcohol, 1 mol. solution, contractility lost in 5 minutes.	In alcohol, 1 mol. solution, contractility lost in 5 minutes.

FIG. 4.—August 22, 1908. Simultaneous Record of Left and Right Sartorius Muscles. Comparative Effects of Ethyl Alcohol (molecular solution in normal saline, 5·8 c.c. per 100) and of Chloroform (centimolecular solution in normal saline, 0·08 c.c. per 100). Temperature = 20°. In the continuation of this experiment, comparisons were made with 0·2 m. ether and 0·02 m. chloroform (not reproduced).

It appears from this experiment that chloroform of centimolecular strength is slightly more effective than alcohol of molecular strength.

From a further experiment made with chloroform at 0·009 m. as compared with alcohol of standard strength, it is found that at this strength chloroform

is considerably less effective. The physiological equality of chloroform with our alcohol standard is between 0·010 and 0·009 m., nearer to the former than to the latter value; we have therefore taken as a sufficiently close approximation that

$$\tfrac{1}{100}\,C = 1\,A \quad \text{or} \quad 100\,A = 1\,C.$$

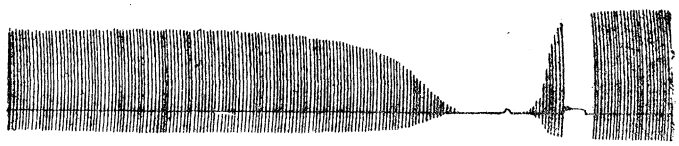
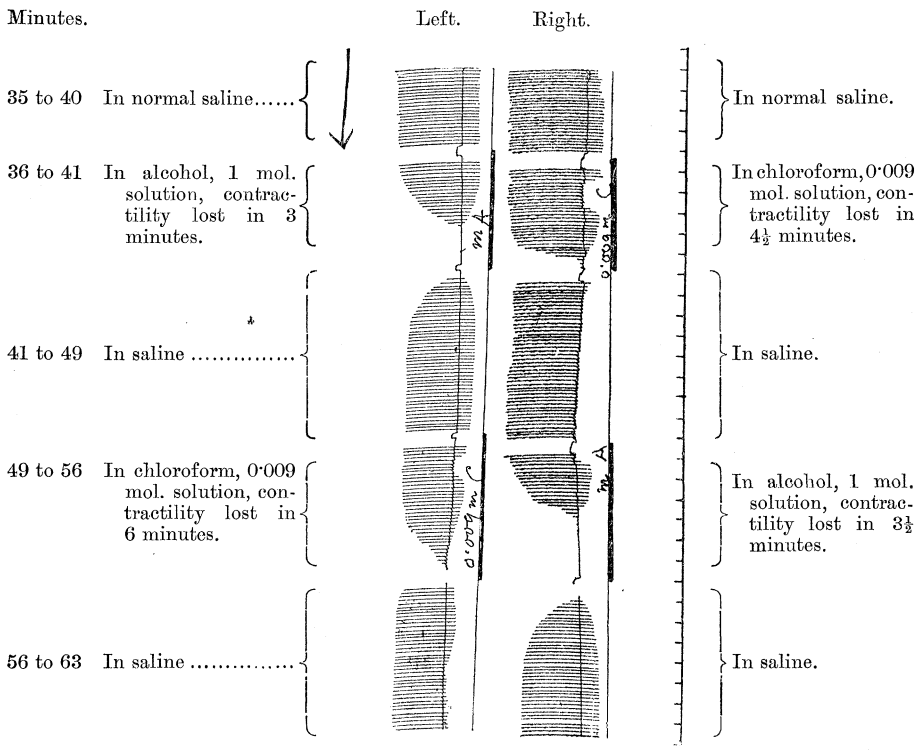


FIG. 5.



(Continuation of record not reproduced.)

70 to 75	In saline	In saline.
75 to 82	In chloroform, 0·009 mol. solution, contractility lost in 5 minutes.	In alcohol, 1 mol. solution, contractility lost in 3½ minutes.
82 to 88	In saline	In saline.
88 to 94	In alcohol, 1 mol. solution, contractility lost in 3 minutes.	In chloroform, 0·009 mol. solution, contractility lost in 5 minutes.

FIG. 6.—August 25, 1908. Simultaneous Record of Left and Right Sartorius Muscles. Effects of Ethyl Alcohol, 1 m. solution, and of Chloroform, 0·009 m. solution. Temperature = 20°.

This relation is not confined to this particular strength. In an experiment with 2 m. alcohol and 0·02 m. chloroform, the two reagents produced substantially equal effects (October 6).

II. *Alcohol* v. *Ether*.

Similar considerations apply to the estimation of the relative physiological efficiency of alcohol and ether.

As compared with a molecular solution of ethyl alcohol it was found—

That a 0·1 mol. solution of ether was too weak,
„ 0·2 „ „ too strong,
„ 0·15 „ „ slightly too strong,

and that the closest approximation to equality of effects was obtained with 0·13 and 0·12 mol. solutions, from which it is concluded that molecule for molecule ether is between seven and eight times as powerful as alcohol.

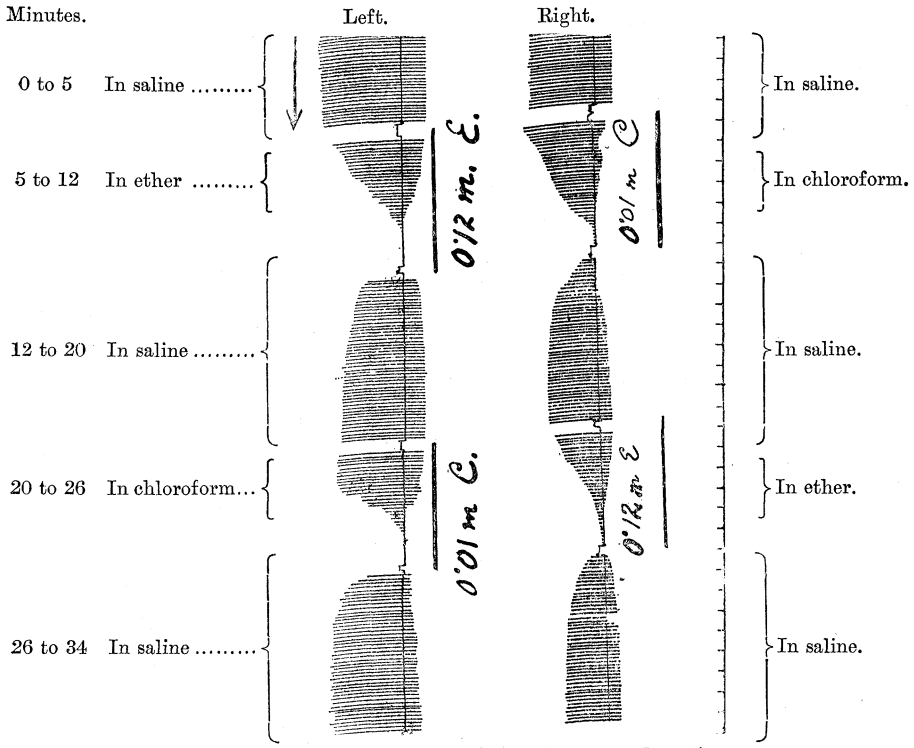
III. *Chloroform* v. *Ether*.

Similar considerations apply to the estimation of the relative physiological efficiency of chloroform and ether.

We commenced by comparing the effects of a 1-per-cent. (by volume) solution of ether and a 1-per-1000 solution of chloroform, these two strengths being respectively equal to 0·1 m. and 0·012 m. The ether solution proved to be considerably the weaker of the two.

For the next trial we took double the strength of ether, viz., 2 per 100 or 0·2 m., and found that at this strength the ether solution was considerably stronger than a chloroform solution of 0·8 per 1000 (= 0·01 m.). In a further comparison between ether, 0·15 m., and chloroform, 0·01 m., the former was still considerably the stronger, and from further trial of 0·13 m. and of 0·12 m. ether we finally determined as nearest to physiological equivalence:

0·01 chloroform = 0·12 ether.



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40 to 45	In saline.....	In saline.
45 to 52	In chloroform, contractility lost in 4½ minutes.	In ether, contractility lost in 6 minutes.
52 to 61	In saline.....	In saline.
61 to 68	In ether, contractility lost in 4 minutes.	In chloroform, contractility lost in 7 minutes.

FIG. 7.—August 26, 1908. Simultaneous Record of the Effects of Chloroform, 0·01 m., and of Ether, 0·12 m., on Two Sartorius Muscles.

The general conclusion from the foregoing experiments is given in the following tabular summary:—

Physiological Equivalence.

	By molecules.	By weight.	By volume.
Alcohol.....	100	100	100
Ether	12	19·3	21·3
Chloroform	1	2·6	1·4

I.e. 1 molecule chloroform = 12 molecules ether = 100 molecules alcohol. *I.e.* a chloroform molecule is 12 times as powerful as a molecule of ether and 100 times as powerful as a molecule of alcohol.

[By weight approximately, 1 gramme of chloroform = 8 grammes of ether = 40 grammes of alcohol.

By volume approximately, 1 c.c. of chloroform = 15 c.c. of ether = 75 c.c. of alcohol.]

Influence of Temperature upon the Rate of Intoxication.

In my earlier experiments upon the rate of intoxication of muscle by alcohol, ether, and chloroform, I paid no particular attention to the temperature beyond noting that the ordinary room temperature during those experiments was comparatively steady at 19° to 21°. But as the degree of precision of which the method was susceptible became apparent, I undertook to examine the quantitative effect of the temperature factor.

The first experiment in this direction (August 18) was made with a 5-per-cent. solution of ethyl alcohol for the purpose of testing the influence of temperature upon the velocity of the reaction between alcohol and muscle upon which the abolition of contractility depends. At 19° muscular contractility was abolished in 7 minutes; at 30° muscular contractility was abolished in $2\frac{1}{2}$ minutes; the velocity of reaction in this case was augmented in very similar degree to the augmentations with raised temperature observed in the saponification of ethyl acetate and in cases of vegetable activity.* In these cases it has been observed that the velocity is increased between twice and thrice with a rise of 10°; in the case of alcohol and muscle the reaction was accelerated nearly threefold by a rise of temperature of 11°.

Similar results were obtained as regards the effect of raised temperature upon velocity of reaction in the case of chloroform and in that of ether.

In the experiment of August 25, the times of abolition of contractility by a 0·02 mol. solution of chloroform (1·6 c.c. per 1000) were—

At 19°	2 min.	and	$2\frac{1}{2}$ min.
28°	0 min. 45 sec.	„	$1\frac{1}{2}$ „

In the experiment of August 27 (fig. 8) the times of abolition of contractility by a 0·15 mol. solution of ether (1·5 c.c. per 100) were—

At 20°	4 min.	and	$4\frac{1}{2}$ min.
28°	$1\frac{1}{2}$ „	„	$1\frac{1}{2}$ „

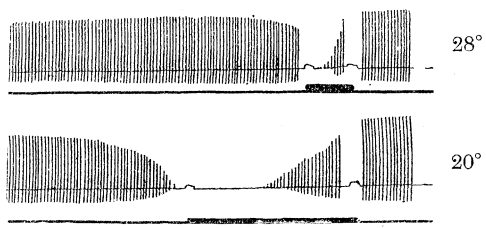

Fig. 8.—August 27, 1908. Effect of Ether Solution, 0·15 m., on a Sartorius Muscle at 20° and at 28°.

* Cohen, 'Beiträge über Physikalische Chemie,' 1901, pp. 37, 43, and 45.

In an experiment of August 28, in which the times of abolition by a 0·01 mol. solution of chloroform (0·8 c.c. per 1000 c.c. saline) were taken by stop-watch, the numbers were noted as 4 minutes at 20°, 2 minutes at 30°, $1\frac{1}{4}$ minutes at 37°.

[*Note* (*added September* 1).—In a report recently presented to Section I of the British Association at Winnipeg, I have brought forward evidence to show that effects of the two anæsthetics, chloroform and ether, are simply additive, *i.e.* the sum of their individual effects.

Taking, *e.g.*, a mixture composed of (1 gramme chloroform + 8 grammes ether) per 1000 c.c. saline, I find that the solution is twice as powerful as a solution of 1 gramme $CHCl_3$ per 1000, or 8 grammes Et_2O per 1000.

Taking, as a point of departure, that 1 c.c. $CHCl_3$ is physiologically equivalent to 15 c.c. Et_2O, I find that the saline solution of a mixture composed of equal volumes of chloroform and ether is approximately half as powerful (actually rather more than half) as the saline solution of a volume of chloroform equal to that of the volume of mixture in solution.

Assuming, as before, that 1 c.c. $CHCl_3$ = 15 c.c. Et_2O, I calculate that the physiological power of a mixture used in clinical medicine composed of two volumes $CHCl_3$ and three volumes Et_2O is 0·27 as compared with the power of chloroform taken = 1·00.

Similarly, that the theoretical value of the well-known A.C.E mixture (one volume alcohol + two volumes chloroform + three volumes ether) referred to the same standard is 0·23.

To put these estimates to the test of experiment, a careful comparison was made of three freshly-prepared solutions, containing respectively—

(1) 2·5 c.c. per 1000 of the mixture (2C + 3E).
(2) 1 c.c. per 1000 of chloroform alone.
(3) 2·5 c.c. per 1000 of the mixture (1A + 2C + 3E).

In correspondence with the fact that the theoretical equivalent amount, in the case of the first solution = 2·3 c.c., and in that of the second solution = 2·7 c.c. (as compared with 1 c.c. in the second or standard solution), it was found that the effect of the first solution came out slightly above that of the standard solution, while that of the third solution came out slightly below that of the standard.]

Note.—Dr. Veley has been kind enough to give me the following calculation, from which it appears that we are really dealing with an alteration of reaction velocity :—

At a temperature of 20° a time 4 min. was required for the abolition of contractility.

" 30° " 2 " " " "

" 37° " 1·25 " " " "

Hence at the end of each minute,

in Case (I) ..... 0·25 unit change took place } Referring to unity,
" (II) ..... 0·5 " " } ratio of numbers
" (III) ..... 0·6 " " } = 1 : 2 : 3·4.

By Esson's formula,* $(\kappa_1 - \kappa) = m \log(\tau_1 - \tau)$.

$\log 2 = 3010$ $\log 3{\cdot}4 = 5051$

$\log 1$

3010 5051

$\log 273+30 = 4814$ $\log 273+37 = 4914$

$\log 273+20 = 4669$ $\log 273+20 = 4669$

0145 0245

$\frac{3010}{0145} = 20{\cdot}8.$ $\frac{5051}{245} = 20{\cdot}6.$ Mean, 20·7.

Hence $0{\cdot}0145 \times 20{\cdot}7 = 0{\cdot}3002 = 1{\cdot}99$ calc., 2·00 found.

$0{\cdot}0245 \times 20{\cdot}7 = 0{\cdot}5072 = 3{\cdot}22$ " 3·20 "

The graph of $\log \kappa'/\kappa$ in terms of $\log \tau/\tau$ is a straight line and is the most convenient form of representation.

* 'Phil. Trans.,' A, 1895, vol. 186, p. 861.