It is satisfactory to find that the results by the carbon-monoxide method agree closely with those hitherto obtained by the aerotonometer method. The reasons why Fredericq and Krogh have obtained no evidence in favour of the secretion theory are also evident. Still more satisfactory is it to find that the process of absorption of oxygen by the lungs is regulated, just as is the breathing itself, in accordance with the physiological requirements of the organism. But for the secretory process the blood would be very incompletely saturated during muscular work, when five, or even ten, times as much oxygen is absorbed as during rest. During rest, on the other hand, the secretory process is not required, and would be a waste of physiological effort.

The Action of Nicotine and other Pyridine Bases upon Muscle.

By V. H. Veley, F.R.S., and A. D. Waller, F.R.S.

(Received February 22,—Read March 10, 1910.)

Nicotine, or pyridyl *n*-methyl pyrrolidine, $C_5H_4N.C_4H_7N(CH_3)$ (m.w. = 162), best known to physiologists in this country in connection with its action on sympathetic ganglia as pointed out by Langley, is generally regarded as having little or no action upon muscle.

According to recent observations of Langley the drug does, however, act upon muscle in a peculiar way, that has led him to a theoretical interpretation which we shall consider later. Our original purpose was the simpler one of comparing upon muscle the action of nicotine and allied substances.

We have made, independently, two separate series of observations, one during September, 1908, with nicotine tartrate, $C_{10}H_{14}N_2.2C_4H_6O_6.2H_2O = 498$, the other during September, 1909, with the free base, following the method described in previous communications, according to which the muscle is excited at intervals throughout observation.

In both series, with differences of detail attributable to the fact that the tartrate in solution is probably to some extent hydrolysed, the nicotine record is unmistakably characteristic, and not presented by any other substance that we have examined. Both in the case of the salt and in that of the base, the drug in moderate concentration produced :—

(1) Contracture with twitching.

(2) A first diminution of contraction not reaching to complete abolition.

(3) A subsequent increase of contraction up to, or even beyond, its original value, the muscle remaining throughout in the nicotine solution.

(4) A final gradual decline of contraction.

Typical records are as under; the "type" is characteristic and reproduces itself in *all* our records.

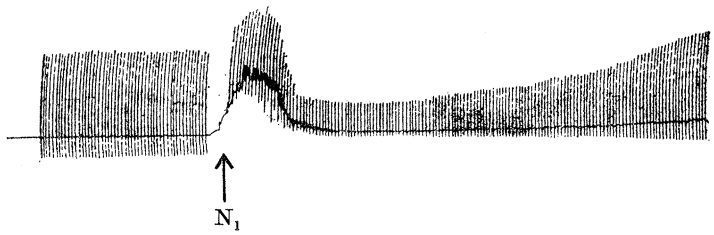


FIG. 1.—Nicotine tartrate, 0·01 per cent. (n/1620 as base); at N_1 a solution of nicotine tartrate, 0·01 per cent., is run in. The typical effect (twitching, contracture, and primary diminished contraction, giving way to increased contraction) is well marked.

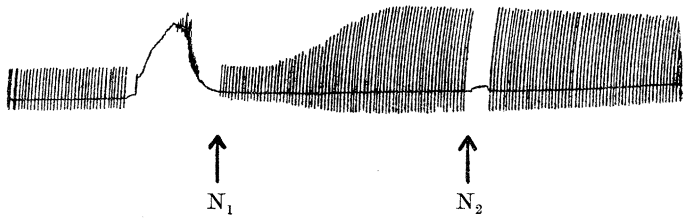


FIG. 2.—Nicotine tartrate, 0·01 per cent. at N_1. Excitation suspended from this point to the next, so as to allow the contracture and twitching to be uncomplicated by the effects of excitation; the subsequent increase of contraction is well marked. 0·1 per cent. at N_2 gives no marked effect.

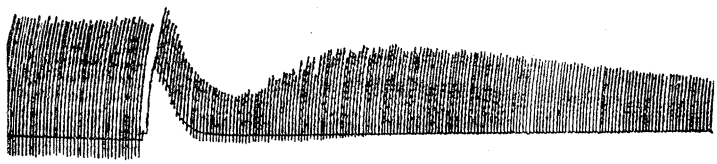


FIG. 3.—Nicotine n/1000 (0·016 per cent.).

Other records (not here reproduced), taken at various strengths of solution, were so much alike as to be hardly distinguishable apart, and altogether different from the records obtained with any other drugs.

Summary of Recorded Observations.

Nicotine Tartrate.

	0·1	per cent.	*n*/162	Typical effect.
	0·1	„	*n*/162	„
	0·2	„	*n*/324	„
	0·01	„	*n*/1620	„ Fig 1.
then	0·1	„	*n*/162	No effect.
	0·01	„	.....	Typical effect.
then	0·1	„	.....	No effect.
	0·01	„	.....	Typical effect.
then	0·1	„	.....	No effect.
	0·01	„	.....	Typical effect (originally small, then large. Fig. 2).
then	0·1	„	.....	No effect.
	0·01	„	.....	Typical effect.
then	0·1	„	.....	No effect.
	0·01	„	.....	Typical effect.
	0·001	„	.....	No effect.
then	0·01	„	.....	Slight typical effect.

Nicotine.

0·081	„	*n*/200	Typical effect; abolition in 11 minutes; imperfect recovery after 20 minutes.
0·041	„	*n*/400	Typical effect; abolition in 15 minutes; fair recovery after 9 minutes.
0·032	„	*n*/500	Typical effect; abolition in 17 minutes; fair recovery after 5 minutes.
0·016	„	*n*/1000.....	Typical effect. Fig. 3.

We find that the peculiar nicotine effect may fail to present itself at lowered temperatures, and that it can be brought out into clearer evidence at higher temperature.

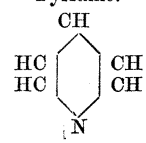
The characteristic nicotine effect cannot be produced twice in the same muscle; if, *e.g.*, it has been brought about by immersion of a muscle in a solution of 0·01 per cent. nicotine, it cannot be reproduced by a second immersion in nicotine solution of ten times the strength (fig. 2), which simply abolishes all muscular contractility.

The characteristic effect fails to appear in the case of muscles removed from frogs that had received by subcutaneous injection a lethal dose of curarine or of nicotine.

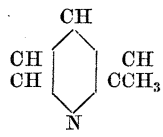
The Pyridine Bases.—The study of nicotine naturally involves that of its parent base pyridine, as well as that of methyl pyridine or picoline and of piperidine or hexahydropyridine, the active principle of pepper.

The constitutional formulæ of these several bases are as follows:—

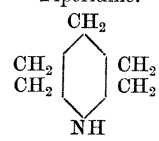
Pyridine.



Picoline.

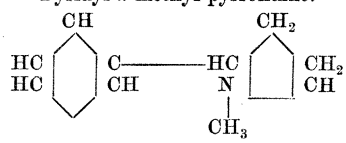


Piperidine.



Nicotine.

Pyridyl *n*-methyl pyrrolidine.



Of these four bases we find that, as regards their action on muscle, nicotine is the most active, and piperidine about half as active as nicotine. Picoline and pyridine have about one-tenth of the toxic power of nicotine. This, of course, is reckoning by molecules.

The characteristic nicotine effect is altogether absent from the records of the three other pyridine bases. To see whether it belongs to the pyrrolidine moiety, or is characteristic of the entire molecule of nicotine, it would be necessary to test the action of the former substance, but so far we have not been able to obtain it.

Summary of Observations.

Piperidine.

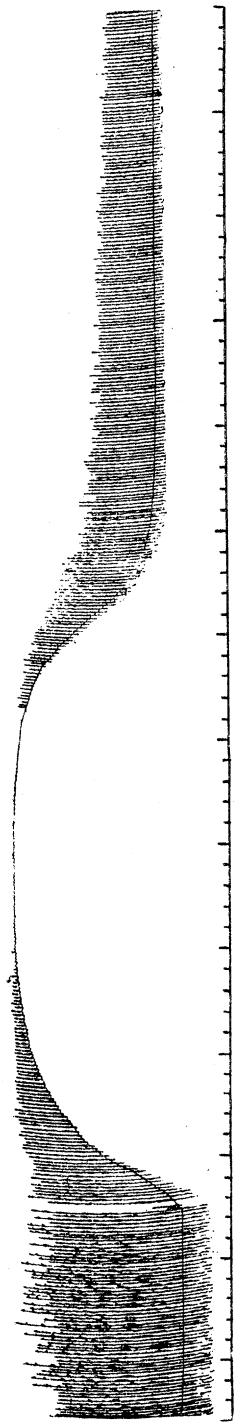
0·085 per cent.		*n*/100.....	Immediate abolition with contracture.
0·021	„	*n*/400.....	Gradual decline. (Fig. 5.)
0·043	„	*n*/200.....	Abolition in 11½ minutes with contracture. Commenced after 12 minutes' imperfect recovery. (Fig. 4.)

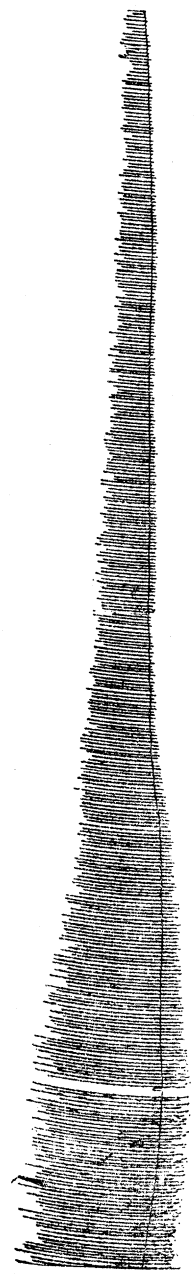
Pyridine. (Fig. 6.)

0·395	„	*n*/20	Abolition in 3 minutes without contracture. Recovery begins in 5 minutes and is complete.
then 0·197	„	*n*/40	No effect.
0·197	„	*n*/40	No effect.
then 0·395	„	*n*/20	Abolition in 5 minutes without contracture.

Picoline. (Fig. 7.)

0·093	„	*n*/100.....	No effect.
0·186	„	*n*/50	Very gradual decline.
0·233	„	*n*/40	Abolition in 13 minutes without contracture; immediate recovery.
0·465	„	*n*/20	Abolition in 5½ minutes; immediate recovery.





FIGS. 4 and 5.—Effect of piperidine upon muscular contraction. Abolition, with contracture, in $n/200$ (0·043 per cent.) solution. Gradual abolition in $n/400$ (0·021 per cent.) solution.

A comparative experiment in which $n/20$ and $n/40$ solutions of pyridine and picoline were alternately applied several times to the same muscle, showed that there was no appreciable difference between the toxic effect of these two bases.

We assign the following order of molecular toxicity to these bases, viz., nicotine = 100, piperidine = 50, pyridine = 10, picoline = 10.

The toxicity of nicotine as compared with that of some other of the poisons that we have examined during the past year can be appreciated from the following numbers, which denote relative molecular toxicity as estimated by experiments on the sartorius muscle:—

Aconitine	1000	Caffeine	12
Quinine.....	100	Chloroform.....	6
Nicotine	33	Ether	0·72
Theobromine.....	18	Alcohol	0·06

Thus, *e.g.*, molecule for molecule, the power of nicotine is about 1/3 that of quinine (or, weight for weight, about 2/3).

Curare and Curarine.—As regards curare and its action upon muscle we made some preliminary experiments, partly for the purpose of meeting an objection made to us that our effects as regards muscle might in part be end-plate effects, but principally in order to see whether curare in weak solution acts directly upon muscle, and to learn whether such action, if present, could be taken as an indication of the specific power of curare tested in the usual way by injection.

We compared two samples of curare. The first, a commercial curare of good repute and price (10*s.* per gramme), but relatively inactive; the second a laboratory preparation of curarine iodide, $C_{19}H_{25}N_2O.I$,* that was given to one of us several years ago by Prof. Boehm, and had been prepared by him from the last consignment of genuine "calabash curare" that was imported into Europe.† This preparation, which is the only derivative of curare exhibiting a crystalline character, has, according to Boehm, the formula $C_{19}H_{25}N_2O.I$; his analyses afforded the following average percentage: C = 53·35; H = 5·85; N = 6·17; I = 30·08.

The relative specific powers of the two preparations may be gathered from the following comparison: a pipette full (0·3 c.c.) of 1-per-cent. solution of

* R. Boehm, "Curare in chemischer und pharmakologischer Beziehung," 'Abh. d. math.-phys. Cl. d. Königl. Sächsischen Ges. d. Wiss., Leipzig,' vol. 22, 1875, 'Das Tubo-Curare'; vol. 24, 1897, "Das Calabassen Curare," "Das Topfcurare."

† This statement is made on the authority of a statement made to me by Prof. Boehm on March 30, 1901.—A. D. W.

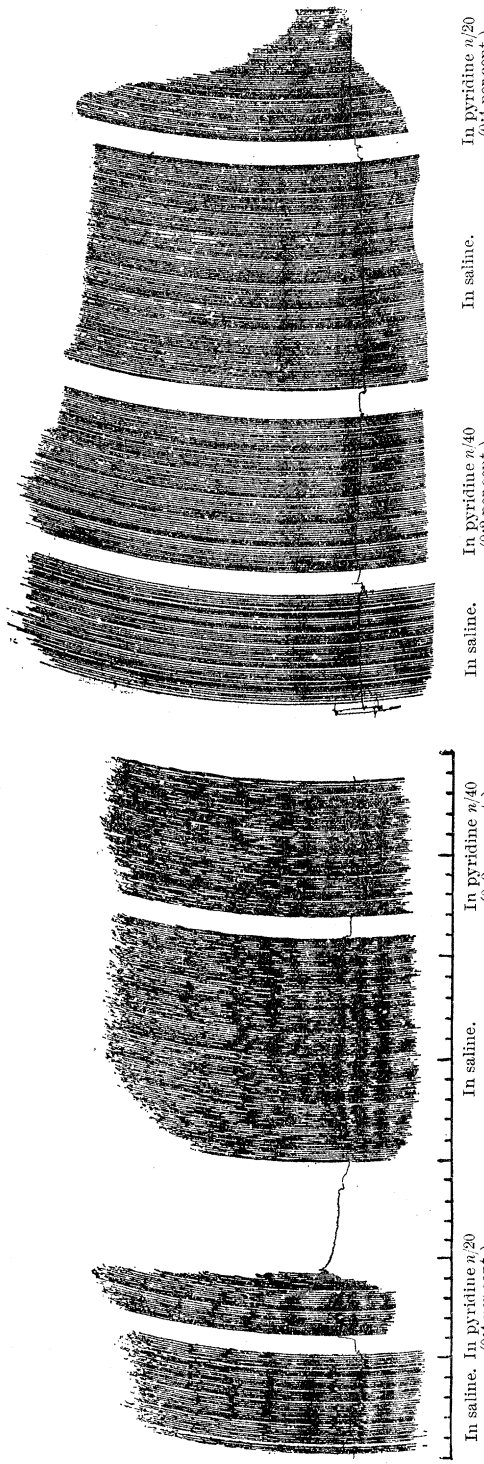


FIG. 6.—Simultaneous record of two muscles, R. and L., immersed in normal saline and in pyridine solutions, as indicated below the record. (The greater amplitude of these as compared with other records is due to the fact that we were then using levers with a greater magnification.)

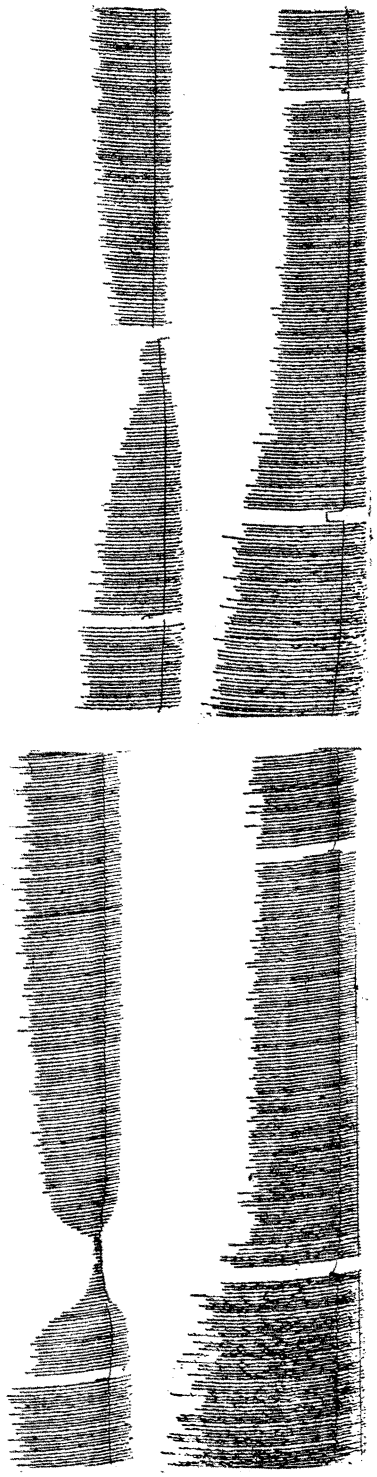


FIG. 7.—Simultaneous record of two muscles, R. and L., immersed in saline and in picoline solutions as indicated on the record.

commercial curare was required for the complete "curarisation" of an average sized frog (20 to 30 grammes); a similar effect, on a similar frog, was produced in a shorter time by the same volume of a 0·01-per-cent. solution of Boehm's preparation.* The latter was thus known to be upwards of 100 times as active as the commercial product, as judged of by its specific action as regards blocking of the impulse from nerve to muscle.

As regards direct effects upon muscle, both preparations of curare were what we are accustomed to call "slightly active," *i.e.* in 0·1-per-cent. solution the contractility of muscle was not abolished in half an hour (fig. 8). Boehm's curarine in 0·1-per-cent. solution was rather more active than the solution of commercial curare at the same concentration (but considerably less active than nicotine at 0·1 per cent. and even 0·01 per cent.); this difference of activity between the two samples of curare was much below the difference as judged by the specific "curarisation" test.

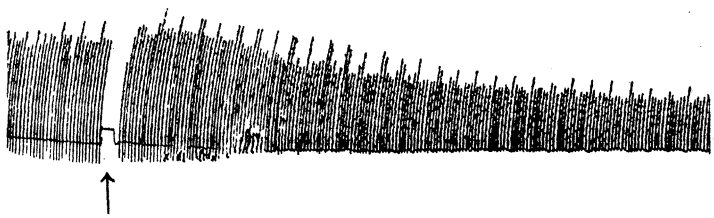


FIG. 8.—Effect of curarine iodide, 1 per 1000, or 0·0024 *n*. (Boehm.)

The Antagonism of Nicotine by Curarine.

Langley has pointed out the antagonism of nicotine by curare; our nicotine and curarine experiments are confirmatory of this antagonism, and bring out approximately a quantitative relation between them. We find by immersing muscles in mixtures of nicotine and curarine solutions in which the proportion of N : C by molecules is varied from 2 : 1 to 160 : 1, that the typical nicotine effect is unfailingly abolished when 30 molecules of nicotine are in presence of 1 molecule curarine. In this instance the nicotine was taken at $n/500$, *i.e.* well above the strength required for a characteristic effect, as, moreover, was indicated by a simultaneous control experiment. The curarine in the mixed solution was at the concentration $n/16000$, *i.e.* far below a concentration at which curarine by itself can act upon muscle. As shown above, curarine in $n/424 = 1/1000$ does not

* Langley's "curare" seems to have been weak, probably the "pot curare" that has replaced "calabash curare" in the market during the last 20 years (R.S., p. 176); he antagonises 4 milligrammes nicotine by 50 milligrammes of curare, and refers to this as being two or three times the amount required to prevent the "sciatic causing contraction of the muscle."

produce abolition of contraction in half an hour, yet, in the mixed solution curarine in 1/40 of that mass, altogether effaces the effect of nicotine. This is a very striking case of antagonism, and as regards the proportion between the antagonistic reagents we have understated it.

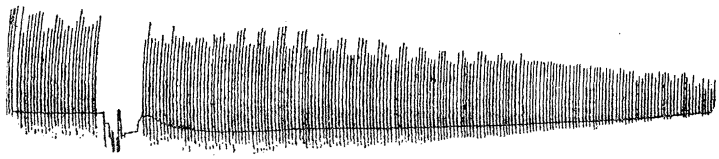


FIG. 9.—Complete abolition of the typical nicotine effect by curarine iodide, N/C = 31/1.

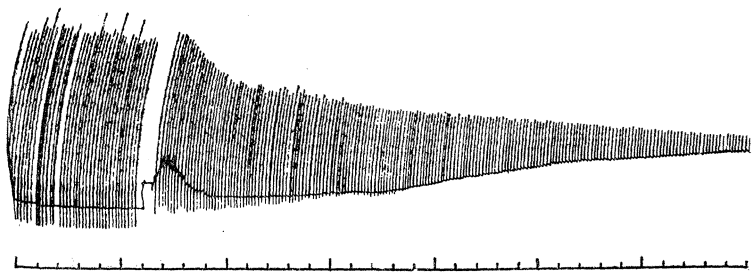


FIG. 10.—Almost complete abolition of the typical nicotine effect by curarine iodide, N/C = 160/1.

Taking the mixed solution with curarine at five times the dilution, *i.e.* with curarine at $n/85000$ and nicotine as before at $n/500$, there is still a very considerable if not absolutely complete abolition of the characteristic nicotine effect. In this case the proportion by molecules is 160 : 1.

That this antagonism is of a peculiar, and, so to speak, specific character, is borne out by experiments with other drugs, where we find that far greater proportional mass is required for the abolition of the characteristic nicotine effect.

We have tested in this connection the influence of cinchonamine. Taking, *e.g.*, a mixture of nicotine at $n/1000$ *plus* cinchonamine $n/2000$, we obtained a record exhibiting the characteristic effects of nicotine and cinchonamine, but in which the nicotine effect was less in the presence of cinchonamine than it would have been in its absence, and in which the cinchonamine effect was lessened by the nicotine. This appears to us to indicate a state of subdivision, the muscle stuff being conjointly occupied by the two bases, nicotine and cinchonamine, as in the ordinary case of subdivision of an acid between two bases; but the case of nicotine and curarine cannot be brought under the same category.

We have also tested mixtures of nicotine and strychnine, with proportions

of nicotine to strychnine = 1 : 2·5 (nicotine $n/1000$ + strychnine $n/400$); the nicotine effect was entirely replaced by the strychnine effect. In the proportion N : S = 4 : 1 (nicotine $n/1000$ + strychnine $n/4000$) the nicotine effect is not completely abolished.

We have also tested mixtures of nicotine and aconitine. Taking a mixture of nicotine $n/1000$ and aconitine $n/40000$ (N : A = 40 : 1), we found that the nicotine effect was absolutely unaltered. With a mixture of nicotine $n/1000$ and aconitine $n/2000$ the nicotine effect was abolished, the record being what we should term a modified aconitine record. With nicotine $n/1000$ and aconitine $n/20000$ the nicotine effect was just visible, while with nicotine $n/1000$ and aconitine $n/10000$ the nicotine effect was abolished.

We also tested mixtures of nicotine and quinine. With a mixture of nicotine $n/1000$ and quinine $n/1000$, we obtained a record indicative of a subdivision, with a considerable predominance of the quinine over the nicotine effect. In the proportion nicotine $n/1000$: quinine $n/5000$ (= 5 : 1) the nicotine effect was not abolished, but in the proportion nicotine $n/1000$: quinine $n/10000$ (= 10 : 1), the nicotine effect is unaltered; that is to say, the effect of 10 molecules of nicotine is not overcome by a molecule of quinine, nor that of five molecules completely so, whereas one molecule of nicotine is overpowered by one molecule of quinine.

Thus in none of these cases could we regard nicotine as antagonised as by curarine. The results were such as to indicate either a subdivision of the muscle protein between two bases or the displacement of a weaker base by a stronger. Whereas in the case of curarine we have a substance with little toxic action on muscle itself, yet of which one molecule was sufficient to very nearly abolish the effect of 160 molecules of nicotine, but it required 1 molecule of strychnine or quinine to overpower 1 molecule of nicotine; and 1 molecule of the most powerful poison we have used, viz., aconitine, could overpower at most 10 molecules of nicotine.

Langley considers that both nicotine and curare combine with some substance in the muscle itself, and that it is unnecessary to resort to the assumption of an additional effect on nerve-endings; he finds, indeed, that the antagonism between these two poisons occurs in muscle of which the nerves have completely degenerated.

We find it difficult, in presence of the fact that curarine (which acts powerfully on nerve-endings, feebly upon muscle) does very readily antagonise the characteristic nicotine effect (the seat of which is now in question), to avoid the conclusion that such nicotine effect takes place at the nerve-ending, and is there antagonised by curarine.

Note 1.—A. V. Hill,* working in Langley's laboratory and according to his method, used extremely dilute nicotine solution, viz., 0·00003 per cent., *i.e.* approximately $n/500000$ (we generally used solutions of 1000 to 500 times this strength, viz. $n/1000$ and $n/500$).

For the antagonism of nicotine by curare, Hill, working with the *rectus abdominis* muscle, gives 0·00006 per cent. (*i.e.* $n/25000$) nicotine as antagonised by a 0·05-per-cent. solution of curare, and 0·00001 per cent. (*i.e.* $n/150000$) as antagonised by a 0·005-per-cent. solution of curare.

We used in our antagonism an $n/500$ (0·03 per cent.) of nicotine with curarine amounting to $n/10000$ (0·0026 per cent.) and $n/85000$ (0·0005 per cent.).

Following the same method, he determines by Arrhenius' formula the temperature constant for nicotine = 17,340, which corresponds with the constant $m = 31·4$ by Esson's formula.

Note 2.—Several years ago, one of us (A. D. W.) made a considerable number of experiments on the isolated frog's heart, for the purpose of comparing the activity of nicotine with that of pyridine. The comparison failed by reason of the irregular effects exhibited by the heart. The failure is worth mention in the present connection in illustration of our opinion that isolated muscle affords a good physiological reagent for the comparison of toxicity. Thus, *e.g.*, the relative power of ether and chloroform can be estimated with far greater accuracy and uniformity of results on isolated muscle than on the isolated heart.

In a recent paper we have given the temperature constants by Esson's formula for

Alcohol ..... $m = 20·8$,
Chloroform ..... $m = 14·3$,
Quinine..... $m = 26·7$;

these correspond with the Arrhenius constants:—11,550, 7930, and 14,820 respectively.

But Hill's paper does not contain any data or records by which it would be possible to compare his results with our own. His conclusion is, however, the same as ours, viz. that the high value of the constant is evidence of a chemical combination between drug and muscle.

Arrhenius' formula, as expressing variation of rate of chemical change with temperature, has been generally adopted by writers on Physical and on Physiological Chemistry.

* 'Journal of Physiology,' vol. 39, p. 361.

It is, however, rather difficult to follow the line of reasoning on which it is based; further, the numbers in the units and the tens of the derived constant μ have no real significance.

On a comparison of the constant m of Esson's equation:—

$$\log K_{T_1} - \log K_{T_0} = m(\log T_1 - \log T_0) \quad (1)$$

with that of μ in the Arrhenius formula, which may be written with the same terms on the left-hand side, thus:—

$$\log K_{T_1} - \log K_{T_0} = \left(\frac{\mu}{2}\frac{T_1 - T_0}{T_1 T_0}\right)\log e \quad (2)$$

(K_{T_1}, K_{T_0} = factors of chemical change at absolute temperatures T_1, T_0 respectively), it appears that $m = \mu/555$, or $\mu = 555\,m$.

The values of the constants m and μ obtained by the two formulæ for the action of the drugs: alcohol, chloroform, quinine (Veley and Waller), and nicotine (A. V. Hill) are here set forth:—

Substance.	Temp. range.	Values of m.	Values of μ.	Ratio.
Alcohol	7° to 24°	20·8	11,570	1 : 556
Chloroform	7° „ 24°	14·3	7,700	1 : 535
Quinine	7° „ 25°	26·7	14,950	1 : 559
Nicotine	17° „ 27°	31·4	17,340	1 : 553

The respective rates at the highest and lowest temperatures respectively have been taken for the purpose of the above comparison; a slight experimental error in one of them would account for the rather low value found in the case of chloroform.

The range of temperature (about 20°) within which the derived constants of the two formulæ are compared may at first sight appear to be rather limited; but this equally applies in practice to most chemical changes which cannot be measured as a rule with any degree of accuracy beyond a limiting range of 30°.