Theoretical Consideration.

Suppose the *whole* liquid to have been well mixed and spread out in a thin layer over N units of area (in the hæmacytometer the usual thickness is ·01 mm. and the unit of area $\frac{1}{400}$ sq. mm.).

Let the particles subside and let there be on an average m particles per unit area, that is Nm altogether. Then assuming the liquid has been properly mixed a given particle will have an equal chance of falling on any unit area.

i.e. the chance of its falling in a given unit area is $1/N$ and of its not doing so $1-1/N$.

Consequently considering all the mN particles the chances of 0, 1, 2, 3 ... particles falling on a given area are given by the terms of the binomial $\left\{\left(1-\frac{1}{N}\right)+\frac{1}{N}\right\}^{mN}$, and if M unit areas be considered the distribution of unit areas containing 0, 1, 2, 3 ... particles is given by $M\left\{\left(1-\frac{1}{N}\right)+\frac{1}{N}\right\}^{mN}$.

Now in practice N is to be measured in millions and may be taken as infinite.

Let us find the limit when N is infinite of the general term of this expansion.

The $(r+1)$th term is:

$$\left(1-\frac{1}{N}\right)^{mN-r} \cdot \left(\frac{1}{N}\right)^{r} \frac{mN(mN-1)(mN-2)\dots(mN-r+1)}{r!}$$

$$=\left(1-\frac{1}{N}\right)^{mN-r} \frac{m\left(m-\frac{1}{N}\right)\left(m-\frac{2}{N}\right)\dots\left(m-\frac{r-1}{N}\right)}{r!}$$

$$=\left(1-\frac{mN-r}{N}+\frac{(mN-r)(mN-r-1)}{N^2 . 2!}-\dots\right.$$

$$\left.+(-1)^s \frac{(mN-r)\dots(mN-r-s+1)}{N^s . s!}+\dots\right)$$

$$\times m \frac{\left(m-\frac{1}{N}\right)\left(m-\frac{2}{N}\right)\dots\left(m-\frac{r-1}{N}\right)}{r!}.$$

But when we proceed to the limit $\frac{1}{N}, \frac{2}{N} \dots \frac{r-1}{N}$ and $\frac{r}{N}, \frac{r+1}{N} \dots \frac{r+s-1}{N}$ are all negligeably small compared to m so that the expression reduces to

$$\left(1-m+\frac{m^2}{2!}-\dots+(-1)^s\frac{m^s}{s!}\dots\right)\times\frac{m^r}{r!}=e^{-m}\times\frac{m^r}{r!}.$$

That is to say that the expansion is equal to

$$e^{-m}\left\{1+m+\frac{m^2}{2!}+\dots+\frac{m^r}{r!}+\dots\right\}.$$

Hence it is this distribution with which we are concerned.

The 1st moment about the origin, O, taken at zero number of particles is

$$e^{-m}\left\{m+\frac{2m^2}{2!}+\frac{3m^3}{3!}+\dots+\frac{rm^r}{r!}+\dots\right\}$$

$$=me^{-m}\left\{1+\frac{m}{1!}+\frac{m^2}{2!}+\dots+\frac{m^{r-1}}{(r-1)!}+\dots\right\}$$

$$=m\times\text{total frequency}.$$

Hence the mean is at m.

The 2nd moment about the point O is

$$e^{-m}\left\{m+\frac{2^2m^2}{2!}+\frac{3^2m^3}{3!}+\dots+\frac{r^2m^r}{r!}+\dots\right\}$$

$$=e^{-m}\left\{m+\frac{2m^2}{1!}+\frac{3m^3}{2!}+\dots+\frac{rm^r}{(r-1)!}+\dots\right\}$$

$$=e^{-m}\left\{m+\frac{m^2}{1!}+\dots+\frac{m^r}{(r-1)!}+\dots+m^2+\frac{2m^3}{2!}+\dots+\frac{(r-1)m^r}{(r-1)!}+\dots\right\}$$

$$=(m+m^2)\times\text{total frequency}.$$

Hence the second moment-coefficient about the mean

$$\mu_2 = m + m^2 - m^2 = m.$$

By similar* methods the moment-coefficients up to μ_6 were obtained, as follows:

$$\mu_1' = m.$$
$$\mu_2 = m.$$
$$\mu_3 = m.$$
$$\mu_4 = 3m^2 + m.$$
$$\mu_5 = 10m^2 + m.$$
$$\mu_6 = 15m^3 + 25m^2 + m.$$

Hence
$$\beta_1 = \frac{\mu_3^2}{\mu_2^3} = \frac{1}{m},$$

and
$$\beta_2 = \frac{\mu_4}{\mu_2^2} = 3 + \frac{1}{m}.$$

It will be observed that the limit to which this distribution approaches as m becomes infinite is the normal curve with its β_1, β_3, β_5, etc., all equal to 0, and $\beta_2 = 3$, $\beta_4 = 15$, etc.

Further, any binomial $(p+q)^n$ can be put into the form $(p+q)^{nq/q}$, and if q be small and nq not large it approaches the distribution just given.

Thus if $1000\,(\frac{99}{100} + \frac{1}{100})^{500}$ be expanded the greatest difference between any of its terms and the corresponding term of $1000\, e^{-5}\left(1 + 5 + \frac{5^2}{2\,!} + \dots + \frac{5^r}{r\,!} + \dots\right)$

* The evaluation of the moments about the point O will be found to depend on the expansion of r^n in the form

$$r^n = r\left\{\frac{(r-1)\,!}{(r-n-2)\,!} + a_1\frac{(r-1)\,!}{(r-n-1)\,!} + a_2\frac{(r-1)\,!}{(r-n)\,!} + \dots + a_{n+1}\frac{(r-1)\,!}{(r-1)\,!}\right\}$$
$$= r\left\{\frac{1}{(r-n-2)\,!} + \frac{a_1}{(r-n-1)\,!} + \frac{a_2}{(r-n)\,!} + \dots + \frac{a_{n+1}}{(r-1)\,!}\right\}(r-1)\,!$$

Then if we form the series for $n+1$ from this it will be found that the following relations hold between a_1, a_2, a_3 etc. and the corresponding coefficients for $n+1$, A_1, A_2, A_3 etc.

$$A_1 = a_1 + n,$$
$$A_2 = a_2 + (n-1)\,a_1,$$
$$A_p = a_p + (n-p+1)\,a_{p-1}.$$

From these equations we can write down any number of moments about the point O in turn, and from these may be found the moments about the mean by the ordinary formulae.

The moments may also be deduced from the point binomial $(p+q)^{nq/q}$ when q is small and n large and $nq = m$, i.e. $p = 1$, $q = 0$, $nq = m$. We have

$$\mu_1' = nq = m,$$
$$\mu_2 = npq = m,$$
$$\mu_3 = npq\,(p-q) = m,$$
$$\mu_4 = npq\,\{1 + 3\,(n-2)\,pq\} = m\,(1+3m) = 3m^2 + m.$$

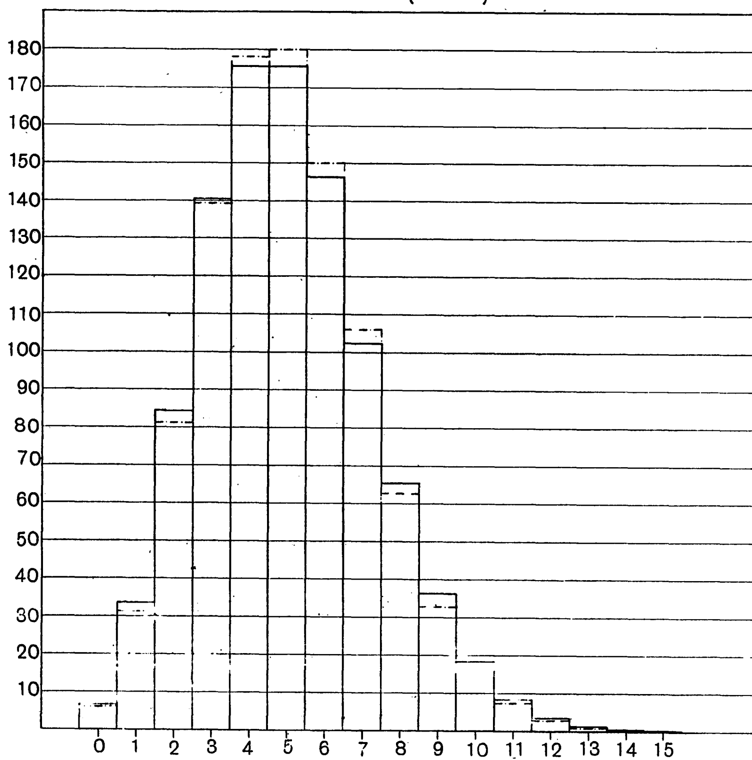
is never as much as 1, being about ·8 for the term $1000\, e^{-5}\,\frac{5^5}{5\,!}$ which is 175·5 against 176·3 from the binomial.

Diagram I compares $1000\, e^{-5}\left(1+5+\frac{5^2}{2\,!}+\ldots+\frac{5^r}{r\,!}+\ldots\right)$ with the binomial $1000\,(\frac{19}{20}+\frac{1}{20})^{100}$ which of course differ, but not by very much.

Diagram I. Comparison of the exponential and binomial expansions.

Firm line represents $1000\, e^{-5}\left\{1+5+\ldots+\frac{5^r}{r!}+\text{etc.}\right\}$.

Broken line represents $1000\left\{\frac{19}{20}+\frac{1}{20}\right\}^{100}$.



In applying this to actual cases it must be noted that we have not taken into account any "interference" between the particles; there has been supposed the same chance of a particle falling on an area which already has several particles as on one altogether unoccupied. Clearly if m be large this will not be the case, but with the dilutions usually employed this is not of any importance.

It will be shewn that the actual distributions which were tested do not diverge widely from this law, so we will consider the probable error of random sampling on the supposition that they follow it.

We have seen that $\mu_2 = m$.

Hence the standard deviation $= \sqrt{m}$.

So that if we have counted M unit areas the probable error of our mean (m) is $\cdot 67449\sqrt{\frac{m}{M}}$.

If we are working with a hæmacytometer in which the volume over each square is $\frac{1}{40000}$ mm. there will be 40,000,000 m particles per c.c. and the probable error will be $40{,}000{,}000 \times \cdot 67449 \times \sqrt{\frac{m}{M}}$.

Suppose now that we dilute the liquid to q times its bulk, we shall then have $\frac{m}{q}$ particles per square, and if we count M squares as before, our probable error for the number of particles per c.c. in the original solution will be $40{,}000{,}000 \times \cdot 67449 \times q\sqrt{\frac{m}{q} \times \frac{1}{M}}$. That is $40{,}000{,}000 \times \cdot 67449\sqrt{\frac{mq}{M}}$.

That is we shall have to count qM squares in order to be as accurate as before.

So that the same accuracy is obtained by counting the same number of particles whatever the dilution, or, to look at it from a slightly different point of view, whatever be the size of the unit of area adopted.

Hence the most accurate way is to dilute the solution to the point at which the particles may be counted most rapidly, and to count as many as time permits: then the probable error of the mean is $\cdot 67449\sqrt{\frac{m}{M}}$ where m is the mean and M is the number of unit areas counted over, squares, columns of squares, microscope fields, or whatever unit be selected.

But owing to the difficulty of obtaining a drop representative of the bulk of the liquid the larger errors will probably be due to this cause, and it is usual to take several drops: if two of these differ in their means by a significant amount compared with the probable error (which is $\cdot 67449\sqrt{\frac{m_1+m_2}{M}}$ where m_1, m_2 are the means and M the number of unit areas counted), it is probable that one at least of the drops does not represent the bulk of the solution.