The virus-containing dog brains proved infective for these mice in doses of 10^{-5} gm. when injected intracerebrally and 10^{-1} gm. when injected intraperitoneally or subcutaneously. Two to 5 intracerebral passages of the virus in these mice reduced the incubation period to 6 days without change in number, size, or appearance of the Negri bodies; the intraperitoneal and subcutaneous titres increased to 10^{-3}. The virus traverses Seitz filters in 10^{-1} and 10^{-2} dilutions when treated in the manner described by Bauer and Hughes.[2]

A mouse protection test for the quantitative measurement of protective antibodies against rabies virus is being developed. Brains from mice prostrate 8 to 9 days after intracerebral injection of mouse brain virus in the 2nd to 5th passage are emulsified, diluted, centrifuged, diluted again, combined with equal parts of test sera for 2 hours at 37°C. and 2 hours at 23°C., and then injected in 0.03 cc. quantities intracerebrally in mice in dilutions of 10^{-1} to 10^{-5}. The duration of life of the injected mice is recorded in days.

Five tests with serum from one individual 1½ years after receiving the last of 3 courses of Semple anti-rabic treatment and with sera from 4 untreated individuals have given uniform results. The protocol of the first test is summarized in Table I. Data thus far show that sera from the 4 untreated individuals do not protect mice against an intracerebral injection of 10^{-6} gm. of mouse brain virus of 4 different strains but that serum from the treated person does protect against at least 100 lethal doses of these same strains.

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Numerical Relations of an Unstable Variant of Salmonella Aertrycke.

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Although many bacteriologists have encountered variants which were difficult to stabilize, most have felt that with sufficient care and repeated selection, any variant could be obtained in a stable form. In our studies on colonial forms of *Salmonella aertrycke* certain variants were encountered which lacked stability. A variant of this

[2] Bauer, J., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

type cannot be obtained free from another variant to which it constantly gives rise. The variation of these cultures was not haphazard but seemed to be governed by some fixed rule, the mechanism of which we have attempted to investigate.

We studied a strain MT2C-R, a rough variant derived from the typical smooth strain of *S. aertrycke*. A typical colony of this variant on infusion agar is low, rough and of medium size. When one was plated on infusion agar about 83% of the colonies derived from it were identical with the parent type. The other 17% were smooth and dome shaped and about 1/10 the diameter of the parent colony (MT2C-S). On continued daily subculture of these rough colonies on infusion agar plates, the percentage of rough colonies remained constant at 83% ± 5%. The S colonies when plated in the same way always yielded 100% S colonies.

When a 24-hour *broth* culture of an R colony was streaked on an agar plate, the percentage of R colonies fell to 33% or less; but each of these R colonies when subsequently plated directly, yielded the original 83%. When, however, the broth culture from the R colony was inoculated into broth a second time the second broth culture, on plating, yielded only S colonies.

Thus the percentage of R forms obtained on streaking out a broth culture of an R colony is less than that obtained by streaking the R colony directly on a second agar plate. Nevertheless, the intermediate broth passage in no way affects the inherent tendency of an R organism to give rise to a colony containing a constant ratio of R and S cells (about 83% R and 17% S).

Although the percentage of R colonies on a plate made from an R colony was found to be constant under constant conditions, a change in the conditions (*e. g.*, pH, temperature, peptone, and the like) gave rise to changes in the percentage of R colonies; but this percentage remained constant at the new level as long as the new conditions were kept constant.

The constancy of behavior of the cultures described above suggested that these phenomena might lend themselves to a simple mathematical analysis.

Let N = total number of cells per unit volume of culture.
R = number of R cells per unit volume of culture.
S = number of S cells per unit volume of culture.
T = time of growth.

Since S cells under the conditions of the experiment never give rise to R cells we may express the rate of increase of R cells during the exponential phase of growth by:

$$\frac{dR}{dT}=CR \qquad (1)$$

S cells, however, may arise from both R cells and S cells so we must write:

$$\frac{dS}{dT}=aS+bR \qquad (2)$$

Where a, b, and c are constants depending on the conditions of growth. $\frac{b}{b+c}$ represents the ratio of the number of S cells derived directly from R cells in an interval dT to the total number of cells derived directly from R cells in that interval.

If equations (1) and (2) are integrated and the integration constants determined by putting:

$$R=R_o \qquad S=S_o \qquad \text{when } T=0$$

we get:

$$R=R_oe^{cT} \qquad (3) \qquad\qquad c=\tfrac{1}{T}\log_e\tfrac{R}{R_o} \qquad (3')$$

$$S=\left(S_o-\frac{bR_o}{c-a}\right)e^{aT}+\frac{b}{c-a}R_oe^{cT} \qquad (4)$$

$$\frac{S}{R}=\left(\frac{S_o}{R_o}-\frac{b}{c-a}\right)e^{(a-c)T}+\frac{b}{c-a} \qquad (5)$$

If $c > a$ then equation (5) gives:

$$\operatorname*{Lim}_{T\to\infty}\frac{S}{R}=\frac{b}{c-a}=\text{a constant} \qquad (6)$$

This is evidently the condition when the organisms are grown on agar as described above. If we start with:

$$\frac{S_o}{R_o}=\frac{b}{c-a}=\operatorname*{Lim}_{T\to\infty}\frac{S}{R}$$

then adding equations (3) and (4) gives:

$$S+R=N=(S_o+R_o)e^{cT}=N_oe^{cT} \qquad c=\tfrac{1}{T}\log_e\frac{N}{N_o} \qquad (7)$$

but for a pure S culture we have:

$$a=\tfrac{1}{T}\log_e\frac{N}{N_o} \qquad (8)$$

then since $\frac{b}{c-a}$ is directly determinable from the final percentage of variants on an agar place we can directly determine the constants a, b, and c for growth on agar.

In broth S/R has been shown to increase as long as T increases, so we must have $a \gtreqless c$. If we assume* $a = b + c$ then adding equations (3) and (4) gives:

$$S + R = N = (S_0 + R_0)e^{aT} = Ne^{aT} \qquad a = \tfrac{1}{T}\log \frac{N}{N_0} \qquad (9)$$

which is the same as we would get from a pure S culture.

Further, equation (5) becomes:

$$\frac{S}{R} = \left(\frac{S_0}{R_0} + 1\right)e^{bT} - 1 \qquad b = \tfrac{1}{T}\log_e\left(\frac{1 + S/R}{1 + S_0/R_0}\right) \qquad (10)$$

It is clear then that the constants can be determined independently in broth and on agar and that it could be possible to check the theory experimentally.

The preliminary experiments give following values for the constants:

	b	a	c	$\frac{b}{b+c}$ (primary ratio)
From S broth	—	1.26 ± .02	1.20 ± .02	.047
" R "	.059 ± .001	1.30 ± .02	1.24 ± .02	.045
" agar	.061 ± .006	1.04 ± .02	1.22 ± .02	.048

Since the experimental error is about 10% the agreement in the values of $\frac{b}{b+c}$ (representing the primary ratio) is probably better than the method allows. As closely as can be determined, this quantity remains a constant, under the different environmental conditions investigated.

We have here an organism which gives rise to R and S forms in a ratio which may be altered by varying the environment, *e. g.,* solid or liquid media. This modification of the ratio depends entirely on the growth rate of the individual daughter types. That is, the environment increases the relative growth rate of one with respect to the other. It in no way alters the primary rate of variation which is a numerical expression of the ability of the cell to give rise to 2 types of daughter cells in constant ratio.

It seems possible that the underlying mechanism of this type of variation is the same as that of the more commonly reported types of dissociation. If the assumptions on which the preceding calcu-

* If $a = c$ (9) becomes $S + R = S_0 e^{aT} + bR_0 e^{aT}$ (9′)
(10) becomes $S/R = S_0/R_0 + bT$ (10′)
The data are not good enough to distinguish finally between these two assumptions but, $a = b + c$ was chosen because of its greater simplicity and a somewhat better check.

lations are based are correct, then we have here a type of culture whose composition may be deliberately changed in opposite directions by varying the environment, *i. e.,* solid or liquid media, without influencing the rate at which new variants arise from the culture. If the commoner forms of dissociation differ only quantitatively from the phenomena here described, then studies on bacterial dissociation must be concerned with 2 distinct phenomena: (1) The rate of origin of the new variant cells (primary rate of variation). (2) The environmental factors which make it possible for the new variant cells to grow to sufficient numbers to be detected in culture.

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Isolation of a Specific Ascorbic Acid (Vitamin C) Oxidase.

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An extract of Hubbard squash oxidizes both the synthetic and the natural ascorbic acid (vitamin C)* with great rapidity. This is due to an enzyme having an optimum pH of 5.83 to 5.96. It may be obtained and purified by extracting the squash (edible part) with twice its weight of 30% ethyl alcohol for 10 minutes. The centrifuged and filtered fluid is treated with an equal volume of acetone, which causes a yellow sticky substance to precipitate. This may be washed free of yellow pigment with acetone, dissolved in water and reprecipitated with acetone. A third precipitation yields a preparation, which after drying *in vacuo* over sulphuric acid has an activity 500 times that of the original extract.

This preparation is water soluble and gives slight protein tests. Alcohol and saturated solutions of neutral salts, however, do not precipitate it. It is digested (inactivated) by trypsin. A polysaccharide accompanies the enzyme in the above precipitation. We have found no way of removing it thus far.

This enzyme differs in various ways from the "hexoxidase" which v. Szent-Györgyi[1] discovered in cabbage leaves, *e. g.,* the

* We are indebted to Professor v. Szent-Györgyi for a sample of l-ascorbic acid and to the Hoffman La Roche Co. for some of their synthetic l-ascorbic acid (Redoxon).

[1] Szent-Györgyi, A., *Science,* 1930, **72**, 125; *J. Biol. Chem.,* 1931, **90**, 385.