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NOTE ON THE DISTRIBUTION OF THERMOPHILIC SPOILAGE BACTERIA

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Spoilage of canned foods by thermophilic sporulating bacteria has been extensively studied by the research staff of the National Canners' Association. In 1920, Donk[7] isolated and described a highly resistant thermophile which withstood a temperature of 100° C. for 17 hours or 120° C. for 11 minutes. In the same year, Bigelow and Esty[2] reported studies in which they showed that the spores of "typical thermophilic organisms" survived an exposure at 120° C. for as long as 22 minutes.

Studies of anaerobic thermophilic bacteria have been reported by Barlow[1] and by Damon and Feirer[6]. Their results are significant to the problem of thermophilic spoilage of foods, but limited space does not permit detailed reference to them here.

After studying 214 cultures of non-gas-forming thermophilic bacteria, Cameron and Esty[4] divided them into two groups, one of facultative and the other of obligate thermophiles. Both types were said to produce the typical "flat sour" condition in canned foods. Extensive studies of the types of thermophilic bacteria which are significant in the high temperature spoilage of canned foods have led to the recognition of three types; namely, the "flat sour," either facultative or obligate thermophilic, and the "hard swell," or those sporulating anaerobic thermophiles which when grown in canned foods liberate sufficient gas to cause the swelling of the container to the familiar "hard swell" deformity. Since the identification of these types, Werkman and Weaver[8] have added a fourth, which is characterized by its ability to liberate large quantities of hydrogen sulfide and for which the name *Cl. nigrificans* is proposed.

The identification of these types of thermophilic bacteria was a long step toward the elimination of the dangers of wholesale spoilage from the category of the difficulties confronting the commercial canner. Cannery surveys by Cameron, Williams and Thompson[5] and by Cameron[3] have demonstrated the effective removal of the seat of infection from cannery systems.

The elimination of these organisms from canneries where spoilage has been encountered is extremely important, but it is equally as significant to determine their natural habitat and, if possible, the means by which they may gain entrance to canning establishments. Such studies must be made in the cannery itself, where the diverse procedures under the various environmental conditions can be observed at first hand. Cameron, Williams and Thompson[5] reported that "Tests for spoilage thermophiles on peas entering the canning plant were uniformly negative. Their presence on raw cut corn was suspected on rare occasions. Tests made upon cane sugar

showed that this substance contained definite numbers of thermophilic spores."

In the preceding references no figures are given to show the extent of the infections. The same workers have since shown the presence of these thermophiles in stable fertilizer and on fresh peas*. With a desire to confirm some of these findings, brief investigations were made at a large corn cannery which offered opportunities for study of the various phases of the problem.

Acknowledgment is due the research staff of the National Canners' Association for cooperation by which the author was enabled to become thoroughly familiar with their methods and with media for cultivation of these spoilage thermophiles. In the present study, two modifications in technique were introduced, the use of beef infusion instead of liver infusion for the cultivation of the "hard swell" thermophiles and the removal of vegetative forms by heating at 80° C. for 20 minutes instead of boiling for 8 minutes.

As practically no spoilage had been encountered in this plant for 10 years, large numbers of the spoilage organisms were not expected. Two series of samples collected in the factory on different days showed few spoilage organisms. Each series included samples obtained from eight different sources in the cannery, beginning with the fresh corn and ending with the canned product. The results showed a total absence of "flat sour" types, the presence of a very few organisms of the *Cl. nigrificans* type, and a predominance of the thermophiles producing hard swells. Two significant points were brought to attention: first, that *Cl. nigrificans* was found only in the preheated, unprocessed corn to which sirup had been added; second, that the time and temperature used to cook the corn were sufficient to kill all the organisms capable of producing hard swells.

The different raw materials entering the plant were comparatively few in number; namely, fresh corn with possibly some soil contamination, sugar, salt, and water. Accordingly, a survey of the possible sources of the two types of spoilage organisms found was made. Samples of four different batches of sugar and salt were examined for spoilage thermophiles, with the following results:

Sugar	(20 samples)	20 contained "flat sour" type
		10 contained *Cl. nigrificans*
		5 contained "hard swell" type

Salt contained no spoilage organisms.

The results obtained from salt are not conclusive, since these organisms are not known to be capable of growth in a salt concentration of from 0.5 to 2.5 percent, which was used in our tests.

Examination of the water used in the cannery revealed no thermophiles of spoilage types.

The results of the examinations of the sugar and salt samples did not indicate a possible source of the large numbers of the "hard swell" type found throughout the factory. Of the materials entering the cannery, the husks removed from fresh corn showed the greatest number of these organisms per gram. Our attention, therefore, was directed to the field soil and its adjuncts. Nine samples of soil and three of manure were obtained from neighboring fields, which were then furnishing the corn to the can-

*Personal communication.

nery. All the fields had been tilled for several years, and most of them had been fertilized more or less continuously.

Seven of the soil samples were from fields previously fertilized with manure and two were from fields fertilized with pea vines. Two of the manure samples were removed from piles which had cured for some months and one was of fresh material. Fifty gram subsamples were thoroughly washed in 100 c.c. of sterile water, and this solution and dilutions thereof were inoculated into the different test media. All media were incubated at 55° C. Of the nine soil samples eight contained the "hard swell" type, six contained *Cl. nigrificans* and none contained the "flat sour" type of thermophiles. Both samples of soil previously fertilized with pea vines contained the "hard swell" type and *Cl. nigrificans* in appreciable numbers, but none of the "flat sour" type. Both samples of cured manure carried the two former types in large numbers, whereas only occasional spores of the two types were found in the fresh material.

Although the numbers of samples examined are too small to justify the drawing of general conclusions, the foregoing results confirm the isolation of *Cl. nigrificans* and the "hard swell" type from manure and indicate their abundance in fertilized soil. The enormous increase in numbers of these types during the curing of manure may be significant in the infection of fields through fertilization.

In a further study of the question of the foci of infection of these organisms in cannery machinery, the results of tests made in another canning factory during the pea packing season should be mentioned. The canned products put out at this place likewise had not shown excessive spoilage for many years. Contrary to expectations, however, large numbers of the "flat sour" type of thermophile were found at several points in the system, and in the finished product. The results of the examination of samples of peas as they underwent the various treatments necessary before processing showed a total absence of the typical "flat sour" type in the fresh product, but an average of 1,000 per gram on peas removed from an accessory blanching tank. As all peas passed through the same filling machines, a large part of the entire output of this factory had become infected with "flat sour" thermophiles, and of 22 cans of the finished product incubated at 55° C., 19 showed "flat sour" spoilage, indicating that most of the season's pack still harbored these organisms in a viable state. An efficient cooling system, which insured the storing of the cans in a well-cooled condition, is all that prevented "flat sour" spoilage on a large scale.

SUMMARY

Studies of the distribution of spoilage thermophiles have shown an abundance of *Cl. nigrificans* and the "hard swell" type in soil and in stable fertilizer. Curing of the manure is believed to increase their number markedly. The "flat sour" type was found only in sugar. Tests in canning establishments have shown that the absence of pronounced thermophilic spoilage of the canned product may or may not be a true indication of the absence of the spoilage thermophiles from canning systems.

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THE FREE ENERGY DECREASE OF ALCOHOLIC FERMENTATION

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The living cell is a special type of energy transformer which obtains its energy by catalyzing certain chemical reactions. It is a matter of importance then, to have a criterion for the amount of energy available to an organism from a given reaction. The criterion commonly used by the biologist has been the heat of reaction, obtained either by the use of heats of formation or heats of combustion of the substances involved. However, the physical chemist has for some time recognized the fact that the heat of reaction is not a criterion of the maximum available energy for the reaction, but that in its stead there must be employed the principle of maximum work or free energy. This issue has been concisely stated by Taylor (1924) to the effect that "The heat of reaction is not the true thermodynamic criterion for chemical reaction. On the contrary, the change of free energy, the capacity of the system to do chemical, electrical or mechanical work will be shown to be the correct measure of the driving force of a reaction. In certain special, but accidental cases the heat of reaction and the free energy of the process may be equal. In the majority of cases this will not be true."

The free energy concept has been applied quantitatively to microbiological reactions only recently, especially by Linhart (1920), Baas-Becking and Parks (1927), Burk (1927) and Buchanan and Fulmer (1928). However, it seemed advisable that there be a summary of the working tools for such calculations and their detailed application to a specific reaction of interest in biology. It is obvious that the discussion in this paper is in no way a substitute for a thorough study of the subject of thermodynamics. Its purpose is merely to point out in some detail typical methods and sources of information for further application.

It is the purpose of this essay, then, to outline very briefly the standard physico-chemical formulations underlying the calculation of the free energy decrease and their application to a specific microbiological process, i. e. to alcoholic fermentation.

This discussion will be taken up in the following order:

I. The available energy of a reaction.
   1. Introduction
   2. At constant volume
   3. At constant pressure
   4. Summary.

II. The calculation of the free energy decrease of a reaction.
   1. Standard concentrations (standard state)
   2. Constant concentrations not standard.

III. The free energy decrease of alcoholic fermentation.
   1. Introduction.
   2. Free energy of formation of CO_2 at various pressures.
   3. The free energy decrease of formation of ethyl alcohol at various concentrations.
   4. The free energy decrease of formation of sucrose solutions.
      (a) From freezing point data
      (b) From vapor pressure data
      (c) The effect of alcohol on the free energy of dilution of sucrose.
   5. The free energy decrease of the reaction, $C_{12}H_{22}O_{11} + H_2O = 4CO_2 + 4C_2H_5OH$ under various conditions as to concentrations of the reacting substances.

IV. General discussion and significance of the free energy calculations for the fermentation reaction.

V. Summary.

I. The Available Energy of a Chemical Reaction.

1. Introduction.

The maximum work which a reacting system is capable of doing, that is the available energy, may be treated under two headings, namely, the reaction takes place at constant volume or it takes place at constant pressure. In the first instance the pressure (P) and in the latter the volume (V) is a variable. The fundamental relationships for the above involve quantities symbolized by A and F respectively. Since these two functions have in several instances been confused a brief review is presented of these quantities and their inter-relationships. Also there will be pointed out the relation between these functions and the heat of the reaction.

2. The Reaction Takes Place at Constant Volume.

The first law of thermodynamics, a statement of the law of conservation of energy, may be formulated as follows:

(1) $A + Q = U$

in which

A = The work content or available energy.
Q = The non-available, "latent", or "bound" energy.
U = The total or intrinsic energy.

The above simply states the fact that of the total energy content of a system (U) a portion may not be available for work, but that such total energy may be divided into that which is available (A) and that not so available or bound (Q)

also,

(2) $A = U - Q$

that is, the available or utilizable energy for performing work is the difference between the total energy and that which is bound.

Now assume that two systems react, then,

(3) $\Delta A = \Delta U - \Delta Q$

in which

ΔA = The increase in available energy
ΔU = The increase in intrinsic or total energy
ΔQ = The gain in heat from the surroundings, i. e. the heat absorbed.

That is, the gain in free energy is the difference between the increase in total energy and the heat absorbed. When external work is done ΔA is negative. If the work done is designated by ΔW, as is often the case, then $\Delta W = -\Delta A$.

Assume that the reaction takes place in such a manner that no work is done by or on the system, i. e. $\Delta A = 0$, and there is no volume change, then, all the change in intrinsic energy will appear as heat, i. e. $-\Delta U = -(\Delta Q)_V$ = the heat of the reaction, or,

(4) $\Delta A = (\Delta Q)_V - (\Delta Q)$

The quantity $(\Delta Q)_V$ is a constant for a given set of conditions while ΔA and ΔQ may vary. The quantity $(\Delta Q)_V$ must not be confused with (ΔQ). This latter quantity by definition is the sum of the gain in available energy and the heat of reaction.

The above relations deal with the first law of thermodynamics and give no clue as to the direction in which the reaction proceeds. The second law of thermodynamics is a formal statement against the production of a perpetual motion machine. In the words of Clausius "It is impossible for a self-acting machine working in a cycle to convey heat from a body at a low temperature to one at a higher temperature; or heat cannot of itself (i. e. without the performance of work by some external agency) pass from a cold to a less cold body."

This is the law of dissipation of energy which means that in practice not all of the intrinsic energy of a system can be converted into external work but that during the reaction some of the energy is "lost" as heat.

This law leads to the generalization that if no external work is done and the volume is constant, that,

(5) $\Delta S = \frac{\Delta Q}{T} = \frac{\Delta U}{T}$

in which ΔS is the entropy increase.

Substituting the value of $T\Delta S$ in Eq (3) we obtain

(6) $\Delta A = \Delta U - T\Delta S$

and in Eq (4)

(7) $\Delta A = (\Delta Q)_V - T\Delta S$

The third law of thermodynamics states that at absolute zero, i. e. $T = 0$, $Q = 0$ and the entropy of pure solids or liquids is zero, or,

(8) $S = \frac{Q}{T}$

Substituting the value of TS for Q in (2) we obtain,

(9) $A = U - TS$

The differentiation of equation (9) gives

(10) $dA = dU - TdS - SdT$

but according to Eq (5) $TdS = dU$ and,

(11) $dA = dU - dU - SdT$

and,

(12) $-S = \left(\frac{\delta A}{\delta T}\right)_v$

Substituting (12) in (9), we obtain,

(13) $A = U + T\left(\frac{\delta A}{\delta T}\right)_v$

and for a system reacting reversibly and isothermally without doing external work.

(14) $\Delta A = (\Delta Q)_v + T\left(\frac{\delta(\Delta A)}{\delta T}\right)_v$

Equation (14) shows that the available energy (maximum work) $(-\Delta A)$ of a reaction and the heat of reaction $(-\Delta Q)_v$ at constant volume are identical only when the available energy does not change with temperature, i. e., when $\left(\frac{\delta(\Delta A)}{\delta T}\right)_v = 0$.

If $\left(\frac{\delta(\Delta A)}{\delta T}\right)_v$ is negative, $-\Delta A > -(\Delta Q)_v$

If $\left(\frac{\delta(\Delta A)}{\delta T}\right)_v$ is positive, $-\Delta A < -(\Delta Q)_v$

That is, values for the heat of reaction at constant volume cannot be assumed to be identical with the available or free energy of a reaction taking place at constant volume.

3. The Reaction Takes Place at Constant Pressure.

In the above development constant volume was assumed. If the volume is not constant the value of A (equation 2) becomes $A + PV$ or,

(15) $F = A + PV = U - Q + PV$

in which F is called the free energy content and is defined as

(16) $F = A + PV$

also,

(17) $F = U + PV - TS$ (from equation 4)

The term $U + PV$ represents the heat content, H, hence the usual formulation,

(18) $F = H - TS$

This is the fundamental equation given by Lewis and Randall (p. 155, 1923).

Also,

(19) $\triangle F = \triangle H - T\triangle S$

for a reaction taking place reversibly and isothermally at constant pressure, hence $-\triangle H$ is the heat of reaction at constant pressure ($\triangle H$ is defined as heat absorbed in the reaction), moreover,

(20) $\triangle F = \triangle U - T\triangle S + P\triangle V = (\triangle Q)_v - T\triangle S + P\triangle V$

It is obvious that the heats of reaction at constant pressure and constant volume are equal only when the volume change is very small; under these conditions $\triangle F = \triangle A$. Differentiation of equation (17) gives,

(21) $dF = dU - SdT - TdS + PdV + VdP$

The value of entropy,

(22) $S = \frac{Q}{T} = \frac{U + PV}{T}$ (If no external work is done)

hence,

(23) $dS = \frac{dU + pdV + VdP}{T}$

(23a) $TdS = dU + PdV$ since $P =$ constant

Substituting the value of TdS in equation (21)

(24) $dF = dU - dU - PdV - SdT + PdV + VdP$

and,

(25) $dF = -SdT + VdP$

for at constant pressure, $dP = 0$

(26) $\left(\frac{\delta F}{\delta T}\right)_P = -S$ (Compare with equation 12)

Substituting in (18)

$$(27)\quad F = H + T\left(\frac{\delta F}{\delta T}\right)_P$$

and for a system reacting isothermally and reversibly,

$$(28)\quad \Delta F = \Delta H + T\left(\frac{\delta(\Delta F)}{\delta T}\right)_P$$ a relation similar to equation (14)

The free energy decrease, $(-\Delta F)$, of the reaction is equal to the heat of the reaction, $(-\Delta H)$, only when the free energy decrease is independent of the temperature, i. e., $\left(\frac{\delta(\Delta F)}{\delta T}\right)_P = 0$,

when,

$\left(\frac{\delta(\Delta F)}{\delta T}\right)_P$ is positive, $-\Delta F < -\Delta H$

and

$\left(\frac{\delta(\Delta F)}{\delta T}\right)_P$ is negative, $-\Delta F > -\Delta H$

4. In Summary, heats of reaction at constant volume or at constant pressure are not the true measure of the available energy under the specified conditions, instead the functions ΔA or ΔF should be used for reaction at constant volume or constant pressure respectively. These two functions are identical only for small volume changes.

The appended list of equations involving the functions A and F arranged in parallel should be useful in summarizing their interrelationships.

TABLE I

Summary of relations involving A and F.

A Constant volume	F Constant pressure
$A = U - Q$ (1)	$F = A + PV = U - Q + PV$ (15)
$A = U - TS$ (9)	$F = U + PV - TS = H - TS$ (17) (18)
$\Delta A = \Delta U - T\Delta S$ (6)	$\Delta F = \Delta U - T\Delta S + P\Delta V$ (20)
$\Delta A = (\Delta Q)_V - T\Delta S$ (7)	$\Delta F = (\Delta Q)_V - T\Delta S - P\Delta V$ (20)
$S = \frac{Q}{T}$ (8)	$S = \frac{U + PV}{T}$ (22)
$A = U - TS$ (9)	$F = U + PV - TS = H - TS$ (17) (18)
$dA = dU - TdS - SdT$ (10)	$dF = dU - SdT - TdS + PdV + VdP$ (21)

$$-S=\left(\frac{\delta A}{\delta T}\right)_V \quad (12) \qquad -S=\left(\frac{\delta F}{\delta T}\right)_P \quad (26)$$

$$A=U+T\left(\frac{\delta A}{\delta T}\right)_V \quad (13) \qquad F=H+T\left(\frac{\delta F}{\delta T}\right)_P \quad (27)$$

$$A=(\triangle Q)_V+T\left(\frac{\delta(\triangle A)}{\delta T}\right)_V \quad (14) \qquad \triangle F=\triangle H+T\left(\frac{\delta(\triangle F)}{\delta T}\right)_P \quad (28)$$

Also, it should be noted that there is much disagreement in the literature with regard to the symbols used for the various thermodynamic functions. In Table II will be found the summary of various important symbologies according to Euken, Jette and La Mer (1925).

TABLE II

Symbols used in thermodynamics.

Function	Conditions of restraint	Authority: Gibbs	Planck	W. McC Lewis	MacDougal	G. N. Lewis	Eucken (1)	Eucken (2)
$U-TS$	T, V	Ψ	F	f	F	A	$-A_T$	$-A_{T,V}$
$U-TS+PV$	T, P	ζ	ϕT	Φ	Φ	F	$-A_m$	$-A_{T,P}$
$U+PV$	T, P	K	—	—	H	H	W	H
$\frac{(U-TS+PV)}{m}$	T, P	μ	—	$\frac{\delta\Phi}{\delta m}$	$\frac{\delta\Phi}{\delta m}$	$\overline{F}$	—	$-A_{T,P}$

(1) 2nd German edition.

(2) Translation by Jette and La Mer.

II. The Calculation of Free Energy Decrease of Reaction.

1. The Standard State.

At a given temperature the value of the free energy decrease ($-\triangle F$) of a reaction depends upon the concentrations of the reacting materials. It is necessary, therefore, to formulate a "standard state" which will be most convenient for general use. The relations involved between $\triangle F$ and "activities" of the reacting materials may be arrived at by the consideration of the reaction,

$$(29) \quad lL+mM+\text{———}=qQ+rR+\text{———}$$

for which,

$$(30) \quad \triangle F-\triangle F^\circ=RT\ ln\frac{\left(a\,{}^{q}_{Q}\right)\times\left(a\,{}^{r}_{R}\right)}{\left(a\,{}^{l}_{L}\right)\times\left(a\,{}^{m}_{M}\right)}-RT\ ln\frac{\left(a^\circ\,{}^{q}_{Q}\right)\times\left(a^\circ\,{}^{r}_{R}\right)}{\left(a^\circ\,{}^{l}_{L}\right)\times\left(a^\circ\,{}^{m}_{M}\right)}$$

in which ΔF and ΔF° represent the free energy of the reaction at the two activities a and a^0 If the "standard state" be defined as that in which the activity of each reacting substance is unity, equation (30) becomes,

$$(31) \quad \Delta F - \Delta F^\circ = RT \ln \frac{\left(a_Q^q\right) \times \left(a_R^r\right)}{\left(a_L^l\right) \times \left(a_M^m\right)}$$

In dilute solutions concentrations may be substituted for activities. Under these conditions the symbol ΔF° represents the free energy of the reaction in the standard concentration (one molal) or at the standard pressure (one atmosphere). If activities need to be employed the standard state is at one molal activity and at a fugacity of one atmosphere. The standard temperature is taken as 25° C. or 298° A. For example, the value for ΔF°_{298} for $\overline{NO}_2$ is 27 Cal., i. e., the free energy decrease of formation per mol of nitrite ion in molal concentration (activity) at 25° is 27 Cal. The free energy decrease of formation of a compound in the standard state is designated by $-\Delta F^\circ_{298}$ and represents the free energy decrease of formation per mol of substance in the particular state prevailing at 25° C.

The value for ΔF for a gas at any fugacity, f_2, may be calculated from the value at any other fugacity, f_1, by the following relation,

$$(33) \quad \Delta F_{f_2} = \Delta F_{f_1} + RT \ln \frac{f_2}{f_1}$$

If the standard be taken as one atmosphere fugacity, then, in large Calories,

$$(34) \quad \Delta F_{298} = \Delta F^\circ_{298} + \frac{RT}{1000} \ln f_2 = \Delta F^\circ_{298} + 1.365 \log f_2$$

For ordinary pressure ranges, as will be shown later, pressures may be substituted for fugacities. For a volatile solute, as ethyl alcohol, the pressures used will be the partial vapor pressures. For example, if ΔF°_{298} represents the free energy increase of formation per mol of alcohol in molal concentration (activity) the value substituted in the standard equations will be the partial vapor pressure of the alcohol at any other molality.

For non-volatile solutes the following relationship holds,

$$(35) \quad \Delta F_{298(a_1)} = \Delta F^\circ_{298} + 1.365 \log a_2$$

in which ΔF°_{298} represents the free energy of formation per mol at molal activity. This value may be calculated as follows. The free energy of dilution is,

$$(36) \quad \Delta F = \frac{-RT}{1000} \ln \frac{a_s}{a} = -1.365 \log \frac{a_s}{a}$$

in which a_s is the activity of the solute in saturated solution. This will have the same value of ΔF as for the pure substance since in a saturated

solution the solid is in equilibrium with the solute. If a be taken as "standard", that is $a = 1$, then,

$$(37) \quad \Delta F^{\circ}_{298} = -1.365 \log a_s$$

While the concentrations may be substituted for activities in equation (35) for usual ranges in biological media this cannot be safely done in equation (37) for in a saturated solution the activity may vary greatly from concentration. This will be illustrated later in the instance of solutions of sucrose.

In Table III are given values of 1.365 $\log a$ for various values of a.

TABLE III

Values of 1.365 $\log a$ for Various Values of a.

a	1.365 $\log a$
2	+0.411
1	0
0.5	−0.411
0.2	−0.955
0.1	−1.365
0.01	−2.730
10^{-3}	−4.095
10^{-4}	−5.460
10^{-5}	−6.825
10^{-6}	−8.19
10^{-7}	−9.55

The use of these relationships may be illustrated by the reaction,

$$\overset{+}{H} + \overset{-}{NO_2} + \tfrac{1}{2}O_2 = \overset{+}{H} + \overset{-}{NO_2}$$

$$0 - 27 - 0 = -0 - 50 + x$$

$$-\Delta F^{\circ}_{298} = 23 \text{ Cal.}$$

that is, the free energy decrease of the reaction per mol of nitric acid formed in molal concentration of nitric acid and of nitrous acid is 27 Cal. It must be noted that the assumption is made that all reacting materials are constantly at molal concentration (activity).

Now assume that the nitric acid is removed from solution in such a manner that its concentration (activity) is constantly 10^{-3} that of the nitrous acid, then,

$$\overset{+}{H} + \overset{-}{NO_2} + \tfrac{1}{2}O_2 = \overset{+}{H}(10^{-3}) + \overset{-}{NO_3}(10^{-3}) + [\overset{+}{H}(0.999m) + \overset{-}{NO_3}(0.999m)]$$

$$0 - 27 - 0 = -4.1 - 50 - 4.1 + x$$

$$-\Delta F_{298} = 31.1 \text{ Cal.}$$

This value is considerably higher than that previously obtained by assuming that the concentrations of the reactants and resultants were

equal. In the above equation the quantities in the brackets represent the concentrations of resultants removed to cause the constant concentration of 10^{-3}.

Again, assume that the concentration of the HNO_2 is 10^{-3} and the HNO_3 constant at 1, then,

$$[\overset{+}{H}(0.999) + \overset{-}{NO_2}(0.999)] + \overset{+}{H}(10^{-3}) + \overset{-}{NO_2}(10^{-3}) + \tfrac{1}{2}O_2 = \overset{+}{H} + \overset{-}{NO_3}$$

$$-\Delta F_{298} = 14.8 - 4.1 - 31.1 = 0 - 50 + x$$

It is obvious from the above numerical examples that the free energy decrease, i. e., the available energy, of the reaction, increases when the concentration of resultants becomes relatively less than that of the reactants and vice versa. If the concentration of HNO_3 were 10^9 that of the HNO_2 the reaction would yield no energy, in fact the value of $-\Delta F_{298} = -1.57$. The oxygen pressure will also have an influence. In the above example it was assumed to be at one atmosphere pressure (fugacity). If the relative concentration of the nitrous and nitric acid did not change, for each tenfold increase in the fugacity of the oxygen the value of $-\Delta F$ will increase 1.36 large calories and will decrease a like amount for each tenfold decrease in pressure.

TABLE IV

Free Energy Decrease of Formation and Heat of Formation of Several Compounds.

[Parks and Huffman (1926); Parks and Kelley (1925); Parks and Anderson (1926); Parks (1925); Lewis and Randall (1923), and various sources.]

Compound	$-\Delta F_{298}$	$-\Delta H$
Methyl alcohol	44.50	61.7
Ethyl alcohol	44.00	69.9
n-Propyl alcohol	44.10	73.8
iso-Propyl alcohol	47.70	80.5
n-Butyl alcohol	44.10	82.8
tert.-Butyl alcohol	49.90	88.4
Ethylene glycol	82.50	113.4
Glycerol	116.70	161.7
Mannitol	226.2	314.9
Dulcitol	228.1	342.9
Glucose	219.1	302.6
Erythritol	152.9	214.8
Formic acid	84.04	102.6
Acetic acid	96.60	117.0
n-Butyric acid	92.5	125.3
Palmitic acid	89.00	214.4
Oxalic acid	167.5	197.6
Acetone	38.00	66.3
Ethyl ether	36.60	70.5
Carbon monoxide (graphite)	32.51	26.1
Carbon dioxide (graphite)	94.26	94.25
Urea	47.28	80.8
Water	56.56	69.0
Formaldehyde	33.0	42.5
*Sucrose	380.0	535.6

*This value was kindly furnished in a private communication by G. S. Parks, who states that the value "is probably good to ± 1%."

The principles outlined above have only recently been employed in the analysis of the available energy of biological processes. One of the early attempts to compare free energy decrease and heats of combustion was made by Baron and Polanyi (1913). These authors studied the oxidation of glucose, of albumin and the transformation of glucose into fats, and found that in general the free energy decrease was from 5-13% greater than the heat of reaction. They made use of an approximate formula of Nernst and employed the chemical constants calculated by this author. Moreover, they employed the heat of formation of water in the liquid state when in fact the reaction involved the formation of water vapor, that is, they neglected the $P\triangle V$ factor. Simon (1922) made more accurate calculations, but still used the Nernst constants which are now considered to be unreliable. The relations developed by Lewis and co-workers have recently been applied to the energy relationships of the autotrophic bacteria by Baas-Becking (1927) and by Linhart (1920) and Burk (1927) to nitrogen fixation.

While these later workers employed concentrations other than standard, in each case they assumed constant concentrations of reacting materials throughout the reaction. In the analysis of the free energy changes in alcoholic fermentation to be outlined, account is taken of the fact of changing concentrations, resultants appearing at the expense of the reactants.

In Table IV are given values for the free energy decrease and heats of formation of several compounds of special interest in bacteriological reactions.

It is apparent that the values for $-\triangle F_{298}$ may differ widely from those for $-\triangle H$. Moreover, there is no appreciable change in the values of $-\triangle F$ in a homologous series while the value of $-\triangle H$ increases regularly with the introduction of the CH_2 group. It follows that the values for $-\triangle F$ for a given reaction, involving members of a homologous series, will increase more rapidly than the values for $-\triangle H$ in ascending the series. Parks and Kelley (1925) generalize further by stating that the substitution of an OH group for a hydrogen leads to a free energy decrease of about 35.5 Cal.

III. The Free Energy Decrease in Alcoholic Fermentation.

1. Introduction.

In the following development we shall assume that sucrose yields only ethyl alcohol and carbon dioxide as represented by the equation,

$$(38) \quad C_{12}H_{22}O_{11} + H_2O = 4C_2H_5OH + 4CO_2.$$

During the course of the reaction the concentration of the reactant is decreasing while the concentrations of the resultants are increasing. These changes all lead to a decrease of available energy for the reaction. It is obvious then that not only cannot standard states be used for calculating the free energy decrease for the reaction, but that variation in concentrations must be taken into consideration. Other factors further complicate the situation; for example, the effect of the salts in the medium, of metabolic products, change of pH and so on, upon the activities of the reacting

materials. Only one of these factors, the effect of the alcohol upon the solubility, and hence upon the activity of the sucrose, will be considered.

2. THE FREE ENERGY OF FORMATION OF CO_2 AT VARIOUS PRESSURES.

The fugacity of a gas may be calculated for a given pressure by means of the following relation,

$$(39) \quad \log f = \log P - \frac{1}{2.303RT}\int_0^P a\,dP$$

in which a represents the difference between the ideal volume (the volume had it obeyed the gas laws) and the actual volume at the given pressure. Values of a were calculated from data given by Landolt-Börnstein. The area under the curve obtained by plotting a against P, from 0 to any value of pressure, P, gives the value of the expression $\int_0^P a\,dP$

In Table V are given values of the fugacity of CO_2 at various pressures as calculated by means of equation (39).

TABLE V

Values for Fugacity of CO_2.

P atmospheres	$\log P$	$\int_0^P a\,dP$	$\frac{1}{2.303RT}\int_0^P a\,dP$	$\log f$	f
1	0	0	0	0	1.
5	0.699	525	0.00915	0.6898	4.896
10	1.000	1,050	0.0183	0.9817	9.587
20	1.301	2,115	0.0369	1.264	18.37
30	1.477	3.190	0.0557	1.421	26.39
40	1.602	4.270	0.0745	1.528	33.7
50	1.699	5,400	0.0942	1.605	40.25
100	2	15,450	0.270	1.730	53.71
200	2.301	26,850	0.468	1.833	68.08
400	2.602	33,700	0.588	2.014	103.3
600	2.778	34,350	0.598	2.180	151.4
800	2.903	32,400	0.567	2.337	217.3

The values for the free energy decrease of formation of CO_2 at various values of fugacity may be calculated by means of equation (34) in which the value of $-\Delta F^\circ_{298}$ is taken as 94.26 Cal. Values of $-\Delta F$ for carbon dioxide at various pressures are found in Table VI.

TABLE VI

Values for $-\Delta F$ for CO_2 at Several Pressures.

P (atm.)	$-\Delta F$ (Cal.)	P (atm.)	$-\Delta F$ (Cal.)
10^{-10}	108.1	20	92.51
10^{-8}	105.4	30	92.29
10^{-6}	102.6	40	92.14
10^{-4}	99.81	50	92.04
10^{-3}	97.04	100	91.86
10^{-2}	95.64	200	91.72
1	94.26	400	91.47
5	93.31	600	91.24
10	92.92	800	91.02

It will be noted that up to 50 atmospheres pressure the use of pressure instead of fugacity makes little difference in the calculated values for $-\Delta F$. It is obvious that any method for removal of the CO_2 during fermentation will increase the available energy and that keeping the CO_2 under pressure will cause the reverse.

3. The Free Energy Decrease of Formation of Ethyl Alcohol at Various Concentrations.

The free energy decrease of formation of ethyl alcohol may be calculated by means of equation (33). Since the alcohol does not attain a high concentration during fermentation the partial vapor pressure may be substituted for the fugacity. Values so calculated from data by Wrewsky (1913) are given in Table VII.

TABLE VII

Free Energy Decrease of Formation of Ethyl Alcohol.

Molal concentration	% alcohol by weight	Molar concent.	Partial P (alcohol)	P/P_0	$(\Delta F^\circ - \Delta F)$ $2.303RT \log P/P_0$	$-\Delta F$
	100		129.8	1	0	45.100
4.94	22.0	4.63	44.6	0.342	—0.636	45.736
3.47	15.9	3.37	37.1	0.286	—0.740	45.840
2.21	10.0	2.16	29.0	0.223	—0.890	45.990
1.10	5.0	1.08	18.0	0.138	—1.170	46.270
0.57	3.00	0.55	12.0	0.092	—1.420	46.520
0.21	1.00	0.21	5.0	0.038	—1.940	47.040
0.21×10^{-1}	10^{-1}	0.21×10^{-1}	0.5	3.8×10^{-3}	—3.300	48.400
0.21×10^{-2}	10^{-2}	0.21×10^{-2}	0.5×10^{-1}	3.8×10^{-4}	—4.670	49.770
0.21×10^{-3}	10^{-3}	0.21×10^{-3}	0.5×10^{-2}	3.8×10^{-5}	—6.035	51.135
0.21×10^{-4}	10^{-4}	0.21×10^{-4}	0.5×10^{-3}	3.8×10^{-6}	—7.400	52.500
0.21×10^{-5}	10^{-5}	0.21×10^{-5}	0.5×10^{-4}	3.8×10^{-7}	—8.765	53.865
0.21×10^{-10}	10^{-10}	0.21×10^{-10}	0.5×10^{-9}	3.8×10^{-12}	—15.590	60.690

4. The Free Energy Decrease of Formation of Sucrose Solutions.

In order to use equation (34) for the free energy of formation of sucrose it will be necessary to calculate the activity of sucrose solutions up to saturation. It was necessary to use three sets of data for these calculations since it was found that the value of a_s varied considerably with the temperature.

(a) *Calculations from Freezing Point Data.*

For these calculations the following relationship was used (Lewis and Randall p. 286),

$$(40)\quad \ln\frac{a_2}{m} = \int_0^m -j\,d\ln m = -j + \int_0^m 0.00057\frac{\Theta}{m}\,d\Theta$$

in which Θ is the freezing point lowering of molal concentration at activity a_2 and,

$$(41)\quad j = 1 - \frac{\Theta}{\lambda m}$$

in which $\frac{\Theta}{\lambda m}$ is the ratio between the given molal lowering of freezing point and λm the molal lowering at infinite dilution, i. e., assuming that the gas law holds.

The value of the first integral (equation 40) which we shall designate as (A) is the area under the curve from 0- m obtained by plotting $\frac{-j}{m}$ against m. The value of the second integral (B) is the area from $0 - m$ under the curve of $0.00057 \frac{\Theta}{m}$ against Θ.

In order to save space the complete calculations are not given. The activity of the sucrose in saturated solution (5 molal) at $-15°$ was found to be 15, i. e., a_s at $-15° = 15$.

(b) *From Vapor Pressure Data.*

The calculations from vapor pressure data may be made by use of the following equation (Lewis and Randall),

$$(42) \quad ln \frac{a_2}{m} = -h - \int_0^m \frac{h}{m} dm$$

in which $h = \frac{55.51\ lna_1}{m} + 1$

and $a_1 = \frac{P}{P_0}$

where P_0 is the vapor pressure of pure water an P that of the solvent in the solution at the given temperature. The value of the integral $\int_0^m \frac{h}{m} dm$ is the value from $0 - m$ of the area under the curve obtained by plotting $\frac{h}{m}$ against m. The value for a_s so calculated was 15.4 at 5.25 molal at 0°, and 19.5 (6.11 molal) at 30°. From these data the value of a_s at 25° was taken to be 18.0. Using this value in equation (34) values for the free energy decrease of dilution were calculated and given in Table VIII, Column 4.

(c) *The Effect of Alcohol Upon the Free Energy of Dilution of Sucrose.*

For a complete analysis of the free energy changes during the reaction account should be taken of the effect of changing concentrations upon the solubility (hence the activities) of the reacting substances. This may be illustrated by the effect of the increase in concentration of the alcohol

TABLE VIII

Free Energy of Dilution of Sucrose.

Molal concentration	$\frac{a_2}{a_s}$	$Log \frac{a_2}{a_s}$	ΔF, 0% alcohol	ΔF, 20%[1] alcohol	ΔF, 50%[1] alcohol
0.001	0.000055	—4.255	—5.810		
0.01	0.00055	—3.255	—4.445		
0.1	0.0055	—2.255	—3.080	—3.02	2.40
0.995	0.066	—1.18	—1.610	—1.41	0.960
1.65	0.124	—0.906	—1.238	—1.04	0.560
2.37	0.206	—0.686	—0.936	—0.720	2.240
3.28	0.337	—0.472	—0.644	—0.440	+0.040
4.12	0.494	—0.306	—0.418	—0.220	+0.280
5.35	0.785	—0.105	—0.143	+0.040	+0.560

[1]These data were not calculated by means of values in columns 2 and 3 but from values of a_2/a_s for 20% an 50% alcohol.

upon the activity of the sucrose. The activity of the sucrose in any concentration of alcohol is,

$$(43) \quad a^1{}_2 = \frac{\text{solubility in water}}{\text{Solubility in the solution}} \times a_2$$

The effect of 20% and 50% alcohol upon the free-energy decrease of solution of sucrose is shown in Columns 5 and 6, Table VIII. It is evident that concentrations of alcohol produced during an ordinary fermentation will not cause enough variation in these values to cause serious error in using the values for pure water.

5. The Free Energy of the Fermentation Reaction.

Form data given in the preceding tables it is possible to calculate the free energy decrease of the reaction,

$$C_{12}H_{22}O_{11} + H_2O = 4CO_2 + 4C_2H_5 = OH$$

in any concentration of sucrose for,

$$(43) \quad -\left(\begin{matrix}\Delta F^\circ{}_{298} + \Delta F \\ \text{sugar} \qquad \text{sugar}\end{matrix}\right) + \left(\begin{matrix}4\Delta F^\circ{}_{298} + 4\Delta F \\ \text{alcohol} \qquad \text{alcohol}\end{matrix}\right) + \left(\begin{matrix}4\Delta F^\circ{}_{298} + 4\Delta F \\ CO_2 \qquad CO_2\end{matrix}\right) - \Delta F \text{ reaction}$$

and,

$$(44) \quad \left(\begin{matrix}-\Delta F^\circ{}_{298} + 4\Delta F^\circ{}_{298} + 4\Delta F^\circ{}_{298} \\ \text{sugar} \qquad \text{alcohol} \qquad CO_2\end{matrix}\right) - \Delta F + 4\Delta F + 4\Delta F \quad -\Delta F \text{ reaction}$$

Since the problem involves the change in free energy of reactions per mol of an initial concentration (M_1) to a final concentration, (M_2), we may write

$$(45)\quad 4\Delta F_{\text{alcohol}} + 4\,\Delta F_{CO_2} - \Delta F_{\text{sugar}} = (\Delta F - K)$$

The expression $(\Delta F - K)$ will give the desired information. Theoretically, at the start with zero concentration of CO_2 and of ethyl alcohol, it is obvious that the free energy decrease of formation at these concentrations approaches infinity. For purposes of simplification we shall assume that the inoculation carries a given small concentration of alcohol. It is also assumed that none of the alcohol leaves the solution. We shall also assume that the medium was originally saturated with CO_2 at one atmosphere of air and that the concentration is constant at that figure, i. e., the partial pressure does not change. This simply assumes free contact with the atmosphere so that mixing is complete. Normally at an atmosphere of air the partial pressure of the CO_2 is 0.003 atmosphere. Using equation (34) the value of ΔF is — 4.800 Cal. and $4\Delta F$ = — 19.200 Cal. We may now write,

$$(46)\quad (\Delta F - K) = 4\Delta F_{\text{alcohol}} - \Delta F_{\text{sugar}} - 19.20.$$

In Table IX are found values of $(\Delta F - K)$ calculated for four initial concentrations of sucrose and three initial concentrations of ethyl alcohol. These data were calculated by means of equation (46).

The meaning of the figures in Table IX may be illustrated by the data in the second column. Assume one molal concentration of sucrose in the presence of 0.21×10^{-3} molal ethyl alcohol, and that CO_2 concentration is constant as previously indicated. The value of $(\Delta F - K)$ under these conditions is — 41.74, that is, the free energy decrease per mol of sugar

TABLE IX.

Values of $(\Delta F - K)$ for Various Concentrations of Sucrose and Alcohol.

Initial EtOH *M*	0.21×10^{-3}				0.21×10^{-1}				0.21			
% by Wt.	0.001				0.1				1%			
Sucrose Initial Molal Concentration	$-(\Delta F - K)$				$-(\Delta F - K)$				$-(\Delta F - K)$			
1.00	41.74				30.80				25.36			
0.90	23.67				23.63				23.03			
0.80	22.52				22.48				22.16			
0.70	21.74	41.40			21.70	30.52			21.54	24.98		
0.60	21.16	23.38			21.12	23.24			21.00	22.64		
0.50	20.60	20.08	41.06		20.10	20.04	30.22		20.54	21.72	24.68	
0.45	.....	.....	24.74		.....	.....	24.70		.....	.....	23.02	
0.40	20.21	21.25	22.93		20.17	21.21	22.89		20.05	21.11	22.29	
0.35	.....	.....	22.23		.....	.....	22.19		.....	.....	21.75	
0.30	19.72	20.60	21.68	40.66	19.20	20.56	21.64	29.82	19.68	20.44	21.32	24.28
0.25	.....	.....	21.20	24.32	.....	.....	21.16	24.08	.....	.....	20.88	22.60
0.20	19.35	19.95	20.79	22.47	19.31	19.91	20.75	22.43	19.31	19.89	20.59	21.83
0.15	.....	.....	20.34	21.62	.....	.....	20.30	21.54	.....	.....	20.10	21.14
0.10	18.68	19.28	19.88	20.96	18.64	19.24	19.84	20.92	18.64	19.12	19.78	20.60
0.05	17.80	18.52	19.08	20.00	17.96	18.48	19.06	19.96	18.00	18.40	18.88	19.68
0.02	17.73	18.14	18.68	19.68	17.69	18.10	18.65	19.54	17.70	18.10	17.94	19.38
0.01	17.60	17.82	18.54	19.24	17.52	17.78	18.53	19.38	17.56	17.94	16.98	19.22

fermented with the above conditions constant is 41.74 Cal. greater than that calculated for standard states. Now assume an initial concentration of sucrose of $0.9M$ in the presence of the amount of alcohol in the solution when the molal concentration of the sucrose has changed from 1 to 0.90. The molality of the alcohol will be $0.4M + 0.21 \times 10^{-3}M$, or for all practical purposes $0.4M$. Under these conditions the value of $(\Delta F - K)$ is -23.7 Cal. The average value, then, of $(\Delta F - K)$ per mol of sucrose changed from $1M$ to $0.9M$ is the average of -41.74 and -23.67 or -32.70. That is, the changing of concentration of the sucrose by fermentation from $1M$ to $0.9M$ has decreased the available energy per mol of sucrose fermented 18.1 Cal.

In Table X are given average values for $(\Delta F - K)$ per mol of sucrose fermented with various initial concentrations of alcohol for a change in molality, (ΔM), of $0.3M$.

TABLE X.

Average Values of $(\Delta F - K)$ per mol of sucrose fermented when $\Delta M = 0.3$.

Initial Sucrose (M)	$0.21 \times 10^{-3}M$	$0.21 \times 10^{-1}M$	$0.21M$
1.00	—27.42	—24.65	—23.02
0.70	—26.52	—23.75	—22.61
0.50	—24.95	—23.36	—22.08

This means that the maximum difference between the free energy decrease of fermentation as calculated for standard states and for conditions likely to obtain in a fermenting mixture is about 27 Cal.

IV. General Discussion and Significance of the Free Energy Calculations for the Fermentation Reaction.

The free energy decrease of the fermentation reaction for components in the standard state may be calculated from data given in Table IV.

$$C_{12}H_{22}O_{11}(S) + H_2O(l) = 4CO_2(l) + 4C_2H_5OH(l)$$

$$-380.0 - 56.6 = -4 \times 94.3 - 4 \times 44.0 + x$$

$$-\Delta F^\circ_{298} = 116.6 \text{ Cal.}$$

Referring again to Table X it is apparent that under the conditions specified the maximum free energy decrease for the conditions specified will be about 23% higher than that calculated for the standard state. Considering the probable inaccuracies in the values in free energy decrease of formation of the compounds as given in Table IV and the fact that the equation assumed for the fermentation does not represent accurately the products formed by biological action, the corrections from the standard state in this case are not of relatively great importance. However, it is worth while, in each instance, in the use of free energy changes of biological processes to analyze the situation along the lines presented above.

Finally it will be of interest to compare the value for the free energy decrease and for the heat of reaction for the fermentation. The heat of reaction is,

$$C_{12}H_{22}O_{11} + H_2O = 4CO_2 + 4C_2H_5OH$$
$$-535.6 - 69 = -4 \times 94.3 \times 69.9 + x$$
$$-\Delta H = 51.2 \text{ Cal.}$$

It is apparent that the free energy decrease of the reaction is two to three times the heat of reaction.

V. Summary.

A review has been presented of the relationships involved in the treatment of energy changes resulting from chemical reactions. The cases treated were those in which the reaction takes place at constant volume or at constant pressure. In the former instance the available energy, or the maximum work which the system is capable of doing, is designated by $-\Delta A$ and in the latter by $-\Delta F$. The fact was emphasized that only under special conditions is the heat of reaction identical with the free energy decrease of the reaction. Hence in dealing with the energy available to an organism from a given chemical change, whenever accurate data are available, the discussion should be based not upon the heat of reaction but upon the free energy function.

The free energy concept was applied to alcoholic fermentation assuming the quantitative relation:

$$C_{12}H_{22}O_{11} + H_2O = 4CO_2 + 4C_2H_5OH.$$

Account was taken of the fact that the concentration of the reactant was continuously decreasing and that of the resultants increasing, both changes leading to a continuous decrease in the available energy of the reaction. In order to simplify the treatment it was assumed that the concentration of the carbon dioxide was constantly in equilibrium with the atmosphere and that there was present in the beginning of the experiment a definite low concentration of alcohol.

When all the above factors were taken into consideration it was found that the free energy decrease of the reaction under conditions likely to obtain in a normal fermentation was about 23% higher than that calculated on the assumption that all the materials were in the standard state. The free energy decrease is two to three times the value of the heat of the reaction.

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THE INHERITANCE OF RESISTANCE TO FOWL TYPHOID IN CHICKENS[1, 2]

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That inherent differences may play an important role in determining resistance to the bacterial diseases among animals has long been recognized, but not until quite recently have any systematic efforts been made to determine the exact part that these differences play. Many factors undoubtedly influence resistance to disease, including environment, general health, the degree of infection, virulence of the infecting organism and others; hence any study attempting to determine the role of inherent variability in resistance to disease must be carefully controlled.

Among the investigators who have reported upon the ability of selective breeding to increase resistance to disease, the results of Webster (1924-25) and of Roberts and Card (1926) are most outstanding. Webster found that the offspring of mice that had withstood an acute infection of mouse typhoid were much more resistant to infection of the same organism than were mice descended from parents that had never been subjected to an attack of this disease. In this work highly inbred lines of mice were used. Roberts and Card have observed a very similar situation in the resistance of chicks to bacillary white diarrhea.

Wright and Lewis (1921) observed marked differences in the resistance of inbred lines of guinea pigs to tuberculosis. One inbred line in particular gave a very high resistance, and when either males or females of this line were crossed with individuals from less resistant inbred lines, or with outside stock, a dominance of the resistance among the offspring was indicated. No apparent relationship between the factors of sex, age, weight at time of infection, rate of growth, and other things that might indicate general vigor was noted.

Hagedoorn, LaBrand and Hagedoorn (1920) have reported upon an inherited difference in resistance to a staphylococcus infection in mice. They attribute the difference in resistance to this infection, observed in albino mice, and susceptibility as observed in Japanese waltzers, as dependent upon one pair of genes.

PURPOSE

In this paper the writers are reporting the preliminary phases of an investigation begun with the purpose of attempting to determine whether or not it would be possible by selection to increase the resistance of chickens

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[1]Paper No. 20 from the Department of Genetics and No. 17 from the Department of Poultry Husbandry, Iowa State College, cooperating.

[2]The writers take this opportunity to express their gratitude to Professors R. E. Buchanan, C. H. Werkman, Charles Murray, G. W. Snedecor and A. E. Brandt for many valuable suggestions. Also to the Bacteriology Department for permission to use much of their equipment.

to fowl typhoid. The term resistance rather than immunity is used, for it is probable that no animal is completely immune to any disease. However, it seems probable that various degrees of resistance toward a given infection exist and if so it should be possible by selection to produce a strain of fowls having a high natural resistance.

METHODS

As foundation stock 219 White Leghorn chickens weighing between two and three pounds were secured and each bird was fed a massive dose of virulent fowl typhoid bacteria, *Shigella gallinarum* (Weldin 1926). Of this number 47.7 percent died (Lambert and Knox—In press). These tests were carried out over a three year interval. At the end of the three years this method of testing was discontinued and the plan of testing baby chicks for their resistance to fowl typhoid was adopted. A far greater number of chickens may be tested in the latter manner with the same equipment and at a much smaller cost, and in a study of this kind it is essential to have large numbers to make the results conclusive[1].

These birds were used as breeding stock and their offspring were subjected as six day old chicks to an inoculation of virulent typhoid bacteria. At the same time chicks of the same age that were hatched from non-tested parents were inoculated, and the chicks were then placed in brooders together. All were allowed an ample supply of feed and water and the conditions were kept as favorable as possible.

Each chick was inoculated intraperitoneally with 12,000,000 organisms suspended in physiological salt solution, a number that had been determined in previous trials to kill about 90 percent of all chicks infected, these chicks of course having as parents non-tested birds. The number of organisms injected was determined by use of the Helber counting chamber, and after counts on a given suspension had been made it was diluted to give the proper dosage. The organism used was grown on veal infusion agar slants for 20 hours at 37° C. In order to insure a uniform virulence the organism was reisolated every third week. Before using a reisolated organism it was always checked for its reaction on sugar media according to the method of May and Goodner (1926).

The Effect of Selection Upon Total Mortality

A total of 1305 chicks were tested with the fowl typhoid organism. The results of these tests are shown in Table I. Of these chicks 410 were descendants of parents that had both survived an acute infection of fowl typhoid. Of this group 168 or 40.9 percent died. Another group of 202 chicks that had as parents a typhoid-surviving male mated with non-tested females gave a mortality of 62.4 percent. Both of the above groups were Single Comb White Leghorns.

All other birds used were from parents not having been tested with the fowl typhoid organism. Of this group 405 were White Leghorns bred similarly to the ones above. Three hundred and fifty-nine or 88.6 percent of them died. A second group of 104 Rhode Island Reds showed a mortal-

[1]Fowl typhoid is primarily a disease of nearly grown or adult birds, but if a highly resistant strain can be produced by selecting for resistance in the baby chicks, this strain can then be tested for their resistance as adults.

TABLE I. The mortality of chicks from parents that had survived an acute infection of fowl typhoid as contrasted with that of chicks whose parents had never been subjected to an attack of this disease.

Group	Parentage of chicks	Total No. inoculated	Total No.[1] dead	Total Pct. dead
I	Non-tested ♂♂ x ♀ ♀ (R. I. R.)	104	102	98.1
II	Non-tested ♂♂ x ♀ ♀ (W. P. R.)	80	66	82.5
III	Non-tested ♂♂ x ♀ ♀ (W. L. x R. I. R.)	104	89	85.6
IV	Non-tested ♂♂ x ♀ ♀ (W. L.)	405	359	88.6
V	Typhoid-surviving ♂ x non-tested ♀ ♀ (W. L.)	202	126	62.4
VI	Typhoid-surviving ♂♂ x ♀ ♀ (W. L.)	410	168	40.9

[1]Mortality from the 3rd to 21st days, inclusive.

ity of 98.1 percent. A third lot of 104 chicks, descendants of White Leghorn males mated with Rhode Island Red females, gave a mortality of 85.6 percent. A group of 80 White Plymouth Rock chicks gave a mortality of 82.5 percent.

The difference between the two groups with typhoid-surviving ancestry, one with double and the other with single, is 21.5 percent. Between the group with double typhoid ancestry and all other White Leghorns from non-tested parents it is 47.7 percent, while between the lot with single typhoid ancestry and all other White Leghorns (non-typhoid-tested ancestry) it is 26.2 percent. All of these differences are very significant.

The probability that differences as great as the ones noted could be due to random sampling has been determined by the X^2 method of Fisher (1925). The value of X^2 for the respective differences is shown in Table II. From this table it will be seen that the odds against differences as great as those noted between the groups with typhoid ancestry and those with non-typhoid-surviving ancestry, being due to chance alone, are inconceivably great. It is also apparent from this table that some causes other than those of random sampling are operative in producing a difference as great as that noted between the lots with single and double typhoid-surviving ancestry.

TABLE II. Probability that differences as great as those noted between the respective groups are due to chance. The parentage of the groups compared is shown in Table I.

Groups compared	I & II	I & III	I & IV	II & IV	III & IV	IV & V	IV & VI	V & VI
Difference in mortality %	15.6	12.5	9.5	6.1	3.0	26.2	47.7	21.5
Value of X^2	13.85	10.82	8.61	2.33	0.74	57.90	202.60	25.09
Probability	*	*	*	0.14	0.41	*	*	*

*Probability against a difference as great as the one noted being due to chance is very great; much less than .01.

Another interesting point to be observed is that the differences between the various breeds are significant. Such differences, however, are probably not representative of the breeds as a whole, but more likely represent strain differences within breeds. More information on this point is desirable.

It is interesting to note in this connection, also, that the chicks from the Rhode Island Red x White Leghorn cross are slightly more resistant than the White Leghorn chicks and much more resistant than the Rhode Island Red chicks. This would seem to indicate a dominance of the factors for resistance carried by the White Leghorn and, likewise, to indicate that the Rhode Island Red may carry factors for resistance that the White Leghorn lacks. The evidence for the last point, however, is not conclusive, since a difference as great as that noted between the White Leghorn chicks with non-typhoid parentage and the crossbred chicks with similar parentage might occur over four times out of ten similar trials ($P = .41$) due to random sampling. These results indicate that multiple factors are operative in determining resistance to fowl typhoid and that different combinations of them exist in the various breeds and strains.

The Effect of Selection Upon the Rate of Mortality

Figure 1 shows the rate of mortality in the various groups of chicks. The percentage of survivors is plotted on successive days after inoculation beginning with the third day, the number of survivors on the third day being considered as 100 percent. Chicks dead before the third day were not considered in plotting the curves because only seven chicks out of the 1305 inoculated died before the third day, and most of these were listed as weak at the time of inoculation. Furthermore, an incubation period is necessary and since the heavy mortality began on the third day the writers felt justified in assuming that any mortality previous to this date was probably due to other causes.

It will be noted that the chicks from typhoid-surviving parents died at a much slower rate than those from non-tested parents. Here also, as in total mortality, the chicks that came from parents which had both survived typhoid infection, died at a slower rate than those that had only one parent that was a typhoid survivor. In these two groups but very little mortality occurred before the fifth day and the mortality was much more gradual than in any of the other groups. The mortality of chicks coming from non-tested parents commenced earlier, namely on the third day, and proceeded at a much faster rate. This indicates a higher potential resistance in the chicks having typhoid ancestry.

Some rather pronounced differences are to be noted in the mortality curves of different breeds as well as in total mortality. The Rhode Island Red chicks proved most susceptible as indicated by both rate of and total mortality. The White Plymouth Rock chicks proved most resistant of the three breeds, while the White Leghorns were intermediate. Crossbred chicks from White Leghorn mated with Rhole Island Red birds gave a slightly less total mortality than the White Leghorns, but their curve of mortality resembled more nearly that of the Rhode Island Red chicks.

The mortality curves of the chicks from the non-tested parents tend to flatten out toward the end of the twenty-first day. This is due mostly to the fact that but few chicks were surviving after the fifteenth day. A similar trend, but less pronounced, may be noted in the curve of mortality of the chicks with typhoid ancestry. In this case, however, many more chicks were living at the end of the fifteenth day, and hence a comparison of the two curves after this period, and even before, is not justifiable.

Mortality after the twenty-first day was not considered as being due to typhoid alone. Most of the chicks surviving to this date had recovered their appetite and activity, and with reasonable care a very large majority of them would have lived to maturity. At the end of three weeks it was necessary to transfer the chicks to poultry houses. The conditions in the latter were very unfavorable, due to crowding and the placing of chicks of different ages in the same house. But in spite of the poor conditions approximately 100 of the chicks grew to maturity.

DISCUSSION

The data presented herein have definitely shown that chicks descended from parents that have survived an acute infection of fowl typhoid have a much higher resistance to the same disease than chicks hatching from birds that have never been subjected to an attack of fowl typhoid. It may be suggested that other causes than inherent resistance are operative here. Two other possibilities present themselves in the group of chicks whose dams had survived the disease. The first of these is that a certain amount of passive immunity may be transmitted to the chick thru the yolk and albumen of the egg, and the other is that the ovary of the hen may be affected, in which case the chick may be infected before hatching and thus acquire a greater resistance.

In consideration of the first of the above possibilities it should be pointed out that in the group of chicks whose sire only was a typhoid survivor this possibility cannot be considered as a cause of greater resistance. The experiments of Smith (1907), Hadley (1914), Learmouth (1923) and others have conclusively shown that acquired immunity is not transmitted to the offspring by their sire. Since this group of chicks had a much higher degree of resistance than chicks from non-tested parents, and intermediate between the latter and the chicks with double typhoid-surviving parentage, some other factors than passive immunity must have been operative in causing this greater resistance.

Relative to the infection of the ovaries of hens that have survived an attack of fowl typhoid, it may be said that there is evidence that this is sometimes the case. Doyle (1926) found infected ovaries in four out of nine hens that reacted to the agglutination test for this disease, but in no case was he able to demonstrate the presence of the bacterium in the eggs from these hens. Likewise, he reports that chicks hatching from eggs from these same hens showed good viability. He further states that the clinical history of outbreaks of avian typhoid in Great Britain present strong evidence against its transmission through the egg. On the other hand, Beach and Davis (1927) have presented evidence which seems to indicate that the disease is transmitted through the egg. In no case did they find the bacterium in eggs laid by hens with infected ovaries, although the particular outbreak of the disease reported by them indicated that this was the manner of its transmission. Granted that transmission through the egg sometimes occurs, it would seem, from evidence presented, to be the exception rather than the rule.

It would appear, therefore, that transmission of the bacterium through the egg cannot have accounted for much of the increased resistance noted in this experiment. If such infection of the eggs were the rule rather than the exception, it is probable that the hatchability of the eggs of the typhoid-

surving birds would have been markedly decreased. Such was not the case, for the hatchability from these birds was, on the whole, very good.

A summary of the separate matings in the group with double typhoid-surviving ancestry, as well as in the other matings, shows a great difference in the mortality of chicks from different males, and this is further reason for not considering these two possibilities as having played any major role in determining the greater resistance of their chicks to fowl typhoid. These data are shown in Table III. It will be noted from this table that the mortality for the males with double typhoid-surviving ancestry varied from 25.8 to 58.7 percent. In the males from non-tested parents it varied from 79.7 to 96.4 percent.

TABLE III. The influence of different sires on the mortality of their chicks when subjected to an acute infection of fowl typhoid.

Parentage of chicks	Sire's No.	No. of chicks	Number[1] dead	Percent dead	Ave. date of death after infection	Breed
Typhoid Surviving						
♂♂ x ♀ ♀	2139	150	88	58.7	11.57	White Leghorn
"	2670	140	55	39.3	11.80	"
"	2570	120	31	25.8	11.65	"
Non-tested ♂♂ x ♀ ♀	18	84	80	95.2	7.35	"
"	19	55	53	96.4	7.47	"
"	22	57	50	87.7	8.63	"
* "	23	125	105	84.5	7.10	"
† "	24	84	71	84.5	7.68	"
* "	23	59	47	79.7	5.25	W. L. x R. I. R.
† "	24	45	42	93.3	5.58	"

[1]Mortality based on the interval from the 3rd to 21st days, inclusive.
*These two groups had the same sire.
†These two groups had the same sire.

The probability for differences as great as the ones noted between the different typhoid-surviving sires being due to chance have been calculated by the X^2 method. The odds against differences as great as those noted between sires 2139 and 2670, and between 2139 and 2570, respectively, are extremely great, while between 2670 and 2570 the odds are also very great (over 43 to 1) against this difference being due to chance alone. According to Fisher, if the odds against any difference being due to chance equal 19 to 1 ($P = .05$) one is justified in assuming that some other cause than chance has produced the difference observed.

Each of these males was mated with a sufficiently large number (approximately 12 each) of females to represent a fair sample of the population. Hence there is little likelihood that infection of the egg previous to the time of laying can have played any great part in causing the marked differences in resistance of the chicks from these males to the fowl typhoid bacterium. Rather, it is probable that these differences are due to variations in the genotypes of the males concerned.

One other point of interest appears in Table III. It will be observed that the average date of survival after inoculation of the chicks from males that were typhoid survivors is much longer than that of the chicks from

males who were not typhoid survivors. It is to be noted, also, that the average date of mortality after inoculation is considerably earlier in the crossbred chicks (White Leghorn x Rhode Island Red). These chicks died on the average in a shorter time after infection than did the White Leghorn chicks from non-typhoid-surviving parents, although their total mortality was less. (See Table I.) This same relationship is shown in another manner in Figure 1.

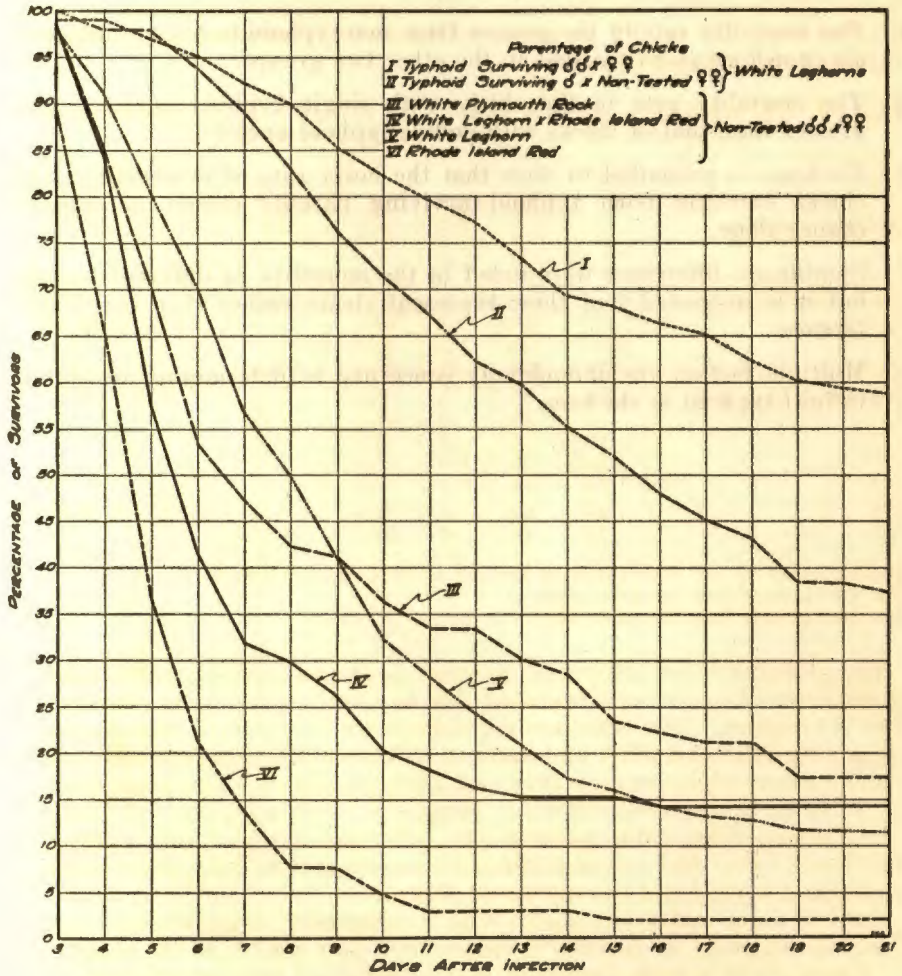

FIG. 1. THE PERCENTAGE OF CHICKS OF THE TOTAL POPULATION SURVIVING ON GIVEN DATES AFTER INFECTION

SUMMARY

1. Chicks hatched from parents that had both survived an acute infection of fowl typhoid gave a total mortality of 40.9 percent.

2. Chicks whose sire only had survived such an infection showed a mortality of 62.4 percent.

3. Chicks from non-tested parents showed a mortality ranging in different breeds from 82.5 to 98.1 percent.

4. The mortality rate in the groups from non-typhoid-tested parents was also much greater than that in the other two groups.

5. The mortality rate in the chicks with single typhoid ancestry was greater than that of chicks with double typhoid ancestry.

6. Evidence is presented to show that the lower rate of mortality in the chicks hatching from typhoid-surviving parents cannot be due to chance alone.

7. Significant differences were noted in the mortality of different breeds, but it is suggested that these represent strain rather than breed differences.

8. Multiple factors are undoubtedly concerned in determining resistance to fowl typhoid in chickens.

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STUDIES IN HOME CANNING*

I. *Some Factors Affecting the Keeping Qualities of Vegetables and Meats Canned by the Hot Water Bath Method***

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INTRODUCTION

Although the hot water bath method of canning vegetables and meats has been employed in the home for many years, a survey of recommended procedures discloses an extreme lack of agreement as regards the period of heating necessary to produce products which will keep satisfactorily. Thus a perusal of bulletins published by the Extension Departments of 21 different states since 1924 indicates the following variations in the heating periods recommended:

Asparagus	1 -3 hours
Beans	1¼-3 hours
Beets	1 -2 hours
Corn	1½-4¼ hours
Greens	1 -3 hours
Peas	1½-3½ hours
Squash	1 -5 hours
Tomatoes	12-45 minutes

The experience with botulism from canned foods has naturally raised a question as to the efficacy of the method formerly recommended for canning both home and commercially prepared products and has led some to look with disfavor on the hot water bath method. Thus the United States Department of Agriculture, Bureau of Home Economics (1924) and Stanley (1926a) recommended that the pressure cooker (autoclave) be used for all vegetables except tomatoes, so as to reduce "spoilage difficulties and the risk of poisoning from occasional contamination with botulinus bacteria." Again Stanley (1926b) stated that "special work has been done on canning asparagus and different varieties of beans and the spoilage records substantiate our earlier conclusion that these vegetables should be processed under pressure," but unfortunately no data were presented to substantiate this conclusion.

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It may be questioned if the steam pressure cooker would be more efficient in the hands of an untrained housewife than the boiling water method with an adequate processing period. The variation in the heating periods recommended for home canning of vegetables has already been referred to. This, together with the scarcity of published data on the efficiency of the water bath method of food preservation, led to the studies reported in this paper.

HISTORICAL

As early as 1909, the United States Department of Agriculture published a bulletin which gave directions and time tables for home canning of non-acid vegetables by the hot water method. (Breazeale, 1909). In spite of the fact that the home canning of vegetables and meats increased tremendously at the time of the war and that time tables for processing by the hot water bath method have been recommended by the U. S. Bureau of Home Economics and the extension workers in the different states, there are very few experimental results published to show the basis for the recommendations made. The available published data on the efficiency of the hot water bath method of canning are reviewed below. No attempt has been made to include the immense canning literature of the commercial field or writing on home canning where experimental data are not given.

Normington (1919) reported results from the canning of 213 jars of peas by the cold pack method, one lot processed in streaming steam, five in the hot water bath and seven at 15 pounds pressure. The proportion of spoilage varied from about 6 to 100% on the different days. Of those autoclaved at 15 lbs., 50.9% spoiled, whereas those cooked 3 hours in the hot water bath showed 63.9% spoilage. On all but one day the peas were left overnight, after gathering, before they were canned. The one time the canning was done on the same day the peas were picked gave a lower percentage of spoilage (12.1% for 40 min. at 15 lbs.). In view of the fact that the freshness of material canned has an important bearing on the keeping qualities, it would be necessary, in comparing the two methods of processing, to restrict the comparison to material of the same degree of freshness. If, therefore, the one batch which was canned fresh is eliminated from the comparison, the proportion spoiled is found to be 63.9% in the hot water bath and 66.7% for that canned at 15 lbs. pressure. These figures are not strictly comparable as some of the processing was done in pint jars and some in quarts and the proportionate number of each was not always given. From the evidence she presented it appears that there was no choice between the pressure cooker and the hot water bath from the standpoint of spoilage.

Skinner and Glasgow (1919)* made an extensive study of the canning of asparagus (664 pints), observing such variables as time of process, different methods of intermittent processing, addition of salt and addition of acid. They found that the 3 hour process without acid showed 50% spoilage, the 2 hour boiling a still greater loss and all processed for less than 2 hours spoiled. They suggested adding one tablespoon of 4.4% vinegar to each quart of water used to fill the jars. With this addition, a 2 hour process was found sufficient for the asparagus to keep.

Margaret MacFarlane (1919) reported results of experiments in can-

*Also reported in Kansas Experiment Station Report, 1919, p. 76.

ning 459 pints of vegetables, using the one period hot water bath process, the intermittent three day process and pressure cooker process at 10 and 15 lbs. With 11 different vegetables and several process methods there were necessarily only a small number of jars of each vegetable canned by each method. In many cases only two or three jars were employed for each variable, the largest number being 40. She recommended intermittent sterilization for asparagus, beans, corn and peas; the one period process 1 hour for beets and cauliflower, 2 hours for beet greens, carrots and Swiss chard, and 22 minutes for tomatoes. The recommendations were based on no spoilage in the following number of jars: asparagus 15, beans 22, beet greens 3, cauliflower 5, corn 15, peas 16, Swiss chard 6, tomatoes 30. In case of beets, 8 of the 26 jars canned by the recommended method spoiled and in the case of carrots there were 2 spoiled out of 25 prepared, but this spoilage was attributed to factors other than length of time of processing.

Biester, Weigley and Knapp (1921) canned 175 jars of vegetables, including beet greens, beets, beans, carrots, corn, tomatoes and pumpkin, by the cold pack method. They noted the effect of storage temperature upon keeping quality. Of 113 jars kept in a cellar at a temperature from 0 to 23° C. only 9 spoiled, while 33 of the 62 jars kept in the incubator at 28° to 32° C. spoiled.

Edmondson, Thom and Giltner (1922), in work with boric acid canning powder, canned one series of vegetables according to the "one period cold-pack" method of Farmers' Bulletin 839. Of the vegetables canned in this way they had spoilage from one of four jars of corn, none of eight asparagus and one of four string beans, none of two lima beans and two of two peas. The others they canned were inoculated with organisms or canned with the addition of canning compound and therefore are not pertinent to this investigation.

Levine (1923), in the course of a series of experiments on the value of boric acid canning compound in food preservation, gave spoilage records for a number of jars canned according to the "cold pack" method of Farmers' Bulletin No. 839, U. S. D. A. Of 31 jars of asparagus, carrots, corn, string beans and green peas processed the recommended time lengths only one jar of corn was spoiled. The temperature of incubation was from 19° to 37° C. Th material was exceptionally fresh, being gathered immediately before canning, the longest time elapsing between picking and the beginning of processing being 3 hours in the case of corn. This might explain the extremely small amount of spoilage.

In 1924 an extensive investigation was carried on with commercial processing of tomatoes. Part of the results would be applicable to home conditions. Esty (1925) reported briefly the results of this work. There were 15,000 cans of tomatoes inoculated with spoilage organisms and processed for different times under different conditions. The temperature and time necessary to sterilize was largely dependent upon the initial contamination. Pressure cooking was thought to offer no advantage over the hot water bath.

Abbott (1926) reported work done on the canning of peas and corn. Two processes were used: 3 hours in the hot water bath and 50 minutes at 10 lbs. pressure. The peas processed by either method spoiled and the corn did not. He stated that the packing method may have been a factor, as the corn was packed very loosely and the jars were filled with hot water, which made the heat penetrate more quickly to the center of the jars.

Considerable work on the value of acid in the canning of vegetables has been done in the laboratory of the University of California by Cruess and his co-workers. Experimental work of Cruess (1916) showed that peas, beans, pumpkins, beets, turnips, artichokes and asparagus processed 1 hour in a brine to which lemon juice had been added kept perfectly, while those without the acid spoiled. He recommended from 4 to 8 oz. lemon juice per gallon of brine for the various products and a process of from 45 minutes to 1 hour in cans and 1 hour to 1½ hours in jars. The details of the experiments were not reported. In a later publication (1925) he recommended 16 tablespoons (8 oz.) strong vinegar or lemon juice per gallon of water used to can asparagus, green beans, beets, carrots, turnips, parsnips and onions and 8 tablespoons for meats, with a process time varying from 2½ hours for beets to 4 hours for most of the vegetables and meats. Although he recommended the same concentration of acid as in 1916, he stipulated a much longer process time than formerly recommended.

Cruess, Fong and Liu (1925) carried on extensive experiments to show the role of acidity in vegetable canning. Their work was done with vegetables inoculated with *Cl. sporogenes, Cl. botulinum,* and a heat resistant thermophile, with brines made up to different pH values by addition of acetic, hydrocloric and citric acids. They found the effect of pH value on heat resistance of these spores to be very pronounced and that it was possible to sterilize canned vegetables much more easily in acidified brines than in non-acidified brines.

Fong (1926) concluded that the final reaction of corn, beans, spinach and asparagus which had been acidified must be pH 5.0 or less to greatly decrease the death time of *Cl. sporogenes, Cl. botulinum* and the thermophile which he employed. When the vegetables were processed for not less than one hour at 212° F., the reaction of the original brine which was found to be dependable for the prevention of spoilage by *Cl. botulinum, Cl. sporogenes,* and the thermophile was pH 2.8 to 3.0. In jars inoculated with *Cl. sporogenes* and processed one hour at 212°, he had no spoilage of sweet corn when the original reaction of the citric acid brine was pH 3.2 and the final pH 5.0, no spoilage of string beans when the original reaction was pH 3.6 and the final pH 5.0, no spoilage in spinach when the original reaction was pH 2.2 and the final pH 4.0, and no spoilage in asparagus when the original reaction was pH 3.6 and the final pH 4.6. With more alkaline reaction or a shorter process time, spoilage resulted.

EXPERIMENTAL

The work reported here is restricted to observations on the hot water bath method, with a view to securing spoilage data and time tables for this method of canning vegetables and meats. The vegetables were canned during the summer of 1926 and 1927 and the meat in the winter of 1927.

The canned products were stored for a period of from 5 to 8 months at temperatures varying from 21 to 29° C. (average about 24° C.). Conditions of canning were controlled as far as was possible. The variety and condition of the product, weather conditions, pH of the water used, barometric pressure and bacterial count of the product as it went into the boiler were noted.

One jar from each boiler was opened as soon as possible after processing to determine the original condition of the product, to serve as a basis

for comparison later. The general appearance, odor, suction, pH. titrable acidity, ammonia and amino nitrogen as determined by the Sörenson formol titration, microscopic examination of the sediment and bacterial counts on plates at 37° C. and 20° C. and in dextrose broth tubes were noted. The jars on the shelves were observed at regular intervals and tests as described above were made on the products as they spoiled. The jars which had not shown evidence of spoilage at the end of the storage period were opened and tested as above.

Jars which gave evidence of bacterial growth or change due to bacterial growth by organoleptic, chemical or bacteriological tests were considered to be spoiled. Jars which did not have a perfect seal at the time of canning, as evidenced by the failure of the new zinc caps to be drawn down by suction, were not included in evaluating the relation of period of processing to spoilage. Such imperfection was found likely to be a cause of spoilage irrespective of the process time.

TABLE I. The record of 3040 jars of vegetables and meats canned during 1926-1927.

	Jars canned	Broken or discarded	Examined† as controls	Jars stored
Pork	288	6	20	262
Beef	336	11	24	301
Tomatoes	144	6	12	126
Asparagus	230	5	18	207
Beans 1926	484	11	39	434
Beans 1927	532*	5	31	472
Chard 1926	120	1	10	109
Chard 1927	264	0	21	243
Sweet Corn 1926	294	3	30	261
Sweet Corn 1927	348	2	29	317
Total	3040	50	234	2732

*24 jars in storage at time of preparing this paper.

†These were opened soon after preparation and examined bacteriologically and chemically to serve as a basis for comparison.

GENERAL PROCEDURE

The variations in the methods followed in the canning of different foods are detailed below. In general, the canning was carried out in the following manner: By previous arrangement with the gardener, the vegetables were gathered and brought immediately to the laboratory. They were thoroughly washed through several waters and cut for packing. The desired amount of vegetable for each jar was weighed and then precooked by adding a measured quantity of boiling water and cooking in a small uncovered sauce pan over a gas burner for five minutes. (*Precooking* is the term used to designate the process whereby the product is thoroughly heated before being put into the jars and the water in which it had been heated is used to fill the jars.) The precooked product was packed into the hot jar as quickly as possible. If the liquid on the product did not fill the jar to within ¼ inch of its top, boiling water was added. A hot cap was screwed on until the seal was within ½ inch of completion. The jars were submerged in sufficient boiling water to cover the caps to a depth of at least three inches and timing was started when the water reached the

boiling temperature. The processing consisted of keeping the jars covered by boiling water for definite lengths of time. After the desired periods of processing, the jars were removed, the seal completed at once, and the jars inverted. They were left in the laboratory overnight and the next morning examined for imperfect seals, labeled and stored. Pint Ball Mason jars were used. New Ball rubbers and new Ball zinc caps were used except where otherwise indicated. Twelve jars were processed at one time in a boiler. Individual racks held the jars.

On the days when the materials were canned, the theoretical boiling point of water varied from 98.87° C. to 99.22° C. due to differences in the barometric pressure.

ASPARAGUS

Methods used.—In the control method with asparagus, the product was scalded and the water discarded, fresh boiling water being used to fill the jars. The scales were removed from the stalks, except at the tip, during the cleaning of the asparagus. Three hundred gram portions of asparagus were used to each pint jar, making a fairly loose pack. Preliminary experiments were made to ascertain the difference in flavor of the asparagus packed cold, precooked and scalded. The asparagus that was scalded and the water discarded was mildest in flavor. Since canned asparagus is considered by some to be too strong in flavor, scalding rather than precooking was chosen for the control method. (Scalding differs from precooking only in that the water used is drained off and discarded and fresh boiling water added to fill the jars.) It was also thought that discarding the scalding water might discard enough bacterial spores to make some difference in the keeping qualities of the product. On one day, half of the jars were filled with scalded asparagus and half with the precooked product. Aluminum caps were used on half the jars in each boiler throughout the canning of asparagus.

The asparagus canned with the addition of acid was treated in the same manner except that the boiling water added to the jar contained 2.0 c.c. of 5 normal phosphoric acid or 1.5 c.c. of 5 normal citric acid for 220 c.c. of liquid used. Preliminary trials indicated that these respective quantities of acid could be added without detrimental effect on the flavor of the product. The 220 c.c. of liquid was added to each jar of scalded asparagus. The reaction of the liquid before processing was pH 3.4 with citric acid and pH 2.2 with phosphoric acid. After processing, the liquid in the case of the non-acidified asparagus showed a pH of 6.0 to 6.2, while that with either citric or phosphoric acid added was pH 5.1. After seven months storage the liquid in the "no acid" jars was pH 5.8-6.0 and that in the acid jars was pH 5.6.

Results.—Asparagus could not always be secured in large enough quantities to can all the jars of one series on the same day. On each day at least ten jars were canned by the control method. There was more variation in the spoilage obtained on different days with these control jars than for the different process times on any single day. For instance, there was no spoilage in the 2 hour process of those canned on June 3d, while on May 11th there was 10% spoilage and May 18th, 78.6%. For this reason the summary of spoilage with different lengths of process must be employed with caution. Apparently some undetermined factors influ-

enced the keeping qualities of the asparagus. The results are summarized in Tables II and IIA.

TABLE II. Showing spoilage records of 207 jars of asparagus canned under different conditions.

Process time in hours			3	2½	2	1½	1
Variable observed	Date canned	No. jars per process time	Percentage spoiled				
Effect of removing scales from stalks							
Scales removed	5/18	14	35.7		78.6		
Scales not removed	5/18	14	35.7		64.3		
Effect of precooking rather than scalding							
Scalded	6/3	10			0		
Precooked	6/3	11			0		
Effect of addition of acid							
No acid (control)	5/14	10		10			
Phosphoric acid	5/14	10, 10, 11			0	0	36.4
No acid (control)	5/23	10	30				
Citric acid	5/23	11, 9, 11			0	11.1	9.1
Effect of batch of material							
Batch canned on	5/11	10			10	50	
* " " "	5/14	10		10			
" " "	5/16	14		28.5		92.9	
* " " "	5/18	14	35.7		78.6		
* " " "	5/23	10	30				
* " " "	6/3	11			0		

*Noted also in sections above.

TABLE IIA. Summary of asparagus canned without acid.

Process time in hours	3	2½	2	1½
Number jars stored	38	24	59	24
Number jars spoiled	13	5	21	18
% spoiled	34.2	20.8	35.6	75.0

Although the removal of the scales from the stalks eliminated considerable dirt, and consequently large numbers of bacteria, such cleansing did not make a significant difference in the percentage of spoilage on the day this factor was used as a variable. From a bacterial count of the dirt washed off in removing scales from two stalks, it was estimated that about 4,000,000 more bacteria were added to the jars when the scales were not removed. However, the scales from the tips were not removed in any case and it is possible that enough dirt was left in them to make the amount removed of no significance.

Precooking, as compared with scalding, made no significant difference in the keeping qualities of asparagus under the conditions that prevailed on the day of the experiment. Bacterial counts of discarded scalding water showed about 500 bacterial spores per c.c. of water. It may be that the plant acids discarded at the same time counterbalanced the advantage of having a product with fewer spores to kill. The pH difference of the two products was not discernable colorometrically, but the titrable acidity was slightly higher in the precooked jars.

The addition of acid had a decided effect on the keeping of asparagus. Both the phosphoric and the citric acid series were run on days when spoilage in the "non-acid" controls showed up in longer process times than were employed for the acid series. There was no spoilage in the 2 hour process when citric acid was added, while the 3 hour "no-acid" series showed 30% spoilage on the same day. There was no spoilage in the 1½ or 2 hour process when phosphoric acid was added, while the 2½ hour "no-acid series of that day gave 10% spoilage.

Since the keeping of asparagus in the 3 hour hot water bath process is partially dependent on some undetermined factor, it would seem advisable to use the 2 hour process with the addition of acid when the hot water bath method is employed.

BEANS

The control method used for canning beans was the same as outlined in the general method for vegetables except that in 1927 the precooking time was shortened to three minutes. For each pint, 340 grams of green

TABLE III. Showing spoilage records of 472 pints of green beans canned under different conditions (1927).

Process time in hours	2½	2	1½	1	
Variable observed	Percentage spoiled*				
Effect of precooking, scalding or packing cold					
Packed cold	0 (10)	0			
Precooked	0	0			
Scalded	0	11.1 (9)			
Effect of delay in processing after precooking					
Processed immediately	9.1		36.4		
Processed after 2 hours	0		0 (12)		
Processed after 4 hours	0 (12)		8.3 (12)		
Effect of storage temperature					
Basement 12-21° C.	0	0			
Room 21-28.5° C.	0	0			
Incubator 37° C.	66.6 (12)	100 (12)			
Incubator 55° C.	100 (12)	100 (10)			
Effect of character of caps and rubbers					
Half aluminum caps, half zinc	0 (23)	0 (23)			
Caps from 1926 spoiled beans	16.6 (12)	25.0 (12)			
Rubbers used once in 1926	0 (10)				
Effect of source of beans					
Secured from B	0 (7)	42.9 (7)			
Secured from H	0 (7)	14.3 (7)			
Effect of consistency of pack					
Loose pack	0	0			
Solid pack	0	0 (10)			
Effect of addition of acid					
No acid added		0	9.1	45.5	
Citric acid added		0	0	0	
Phosphoric acid added		0	0	9.1	

*Percentage based on 11 jars except where otherwise indicated by numbers in ( ).

beans were used in 1926, 300 grams of yellow beans in 1926 and 275 grams of green beans in 1927. The beans canned in 1926 were stored for a period of about eight months and those canned in 1927 for five to six months. The variables, aside from time of processing, were: various methods of "pretreatment" (scalding, precooking or packing cold), delay in processing after precooking, storage temperatures, caps and rubbers, source of product, tightness of the pack, addition of acid, delay in completing the seal, and freshness of the product. The results are summarized in Tables III, IV and IVA.

TABLE IV. Showing spoilage records of 434 pints of beans canned in 1926 under different conditions.

Process time in hours	4	3½	3	2½	2	1½
Variable observed	Percentage spoiled*					
Effect of storage before canning						
Canned fresh	0	0	4.5	0 (10)	22.7	70 (10)
Canned after 1 day	9.1	33.3 (21)	13.6	30 (10)	18.2	54.5 (11)
Canned after 3 days	0 (18)	9.1	13.6	36.4 (11)	42.9 (21)	27.3 (11)
Canned after 5 days	18.2	28.6 (21)	59.1	100(5)	73.3 (15)	
Effect of time elapsing before completion of seal						
Seal completed after 15 sec.	0 (4)					
Seal completed after 30 sec.	0 (4)					
Seal completed after 45 sec.	0 (4)					
Seal completed after 60 sec.	0 (16)					

*Percentage based on 22 jars where not otherwise indicated by number in ( ).

TABLE IVA. Summary of beans canned under ordinary conditions.*

(From Tables III and IV)

Process time in hours	4	3½	3	2½	2	1½	1
Number jars stored	50	22	22	144	144	32	11
Number jars spoiled	0	0	1	1	10	12	5
% spoiled	0	0	4.5	0.6	6.9	37.5	45.5

*Excludes those stored at incubator temperature, those treated with acid, those canned with caps from spoiled beans and those stored before or after precooking before processing.

Effect of scalding, precooking or packing cold.—To ascertain the effect of methods of treatment before processing the following series were prepared:

(1) 24 jars were filled with beans which had been precooked for 3 minutes. These served as controls.

(2) 24 jars were prepared with beans that had been scalded for the same length of time. The water had been discarded and the jars were filled with boiling water.

(3) in 24 jars the beans were packed cold and covered with boiling water.

Scalding, precooking or packing cold made no significant difference in the keeping of the beans.

Effect of delay in processing after precooking.—To ascertain the effect of delay in processing, 24 jars were processed immediately after precooking, 24 were allowed to stand in a warm room (at about 26° C.) for two hours and another 24 for four hours. A temperature record was taken of the cooling curve of the beans after precooking. At the end of one hour the temperature was 52° C., at the end of two hours 39° C., and at the end of three hours 31° C. The heating curves of the cooled beans and the beans processed while hot were not taken as Magoon and Culpepper (1922) have shown that water bath temperature was reached only six or seven minutes later when quart jars of string beans were started at 20° C. than when they were started at 80° C. The difference would be still less in pint jars.

The bacterial count taken from one jar of the beans changed, on standing, from 4 per c.c. in the precooked material to 25 per c.c. in the same after two hours and to 40 per c.c. after four hours. The two and four hour liquids, after being brought to boiling, gave a count of 8 and 3 per c.c., respectively.

The delay in processing after precooking had a favorable effect on the keeping of the beans. The beans that were held for two hours after precooking before processing showed no spoilage in the 2½ or 1½ hour process, while those that were processed immediately showed 9.1% and 36.4% spoilage, respectively. This might appear at first thought contrary to our usual conceptions. These results might be explained in the following manner. The vegetative cells were killed, for the most part, by the precooking. Many of the spores might germinate during the time elapsing before beginning the final processing, thus reducing the number of resistant cells to be destroyed. Similar experiments would have to be carried out with beans on different days and beans from many sources before one could say that holding for a definite interval after precooking would be likely to reduce spoilage. The number of jars canned was not large enough to warrant definite conclusions, but the results indicate that experiments along this line might be illuminating.

Effect of storage temperature.—The beans that were to be stored at different temperatures were canned according to the control method. Three jars from each boiler were taken to make up the 24 stored at each temperature range. Those stored in a basement were in a private home in the vegetable room opening from the furnace room. The temperature in August was as high as 21° C. in this basement room and the lowest temperature recorded was 12° C. Those stored at room temperature were subjected to a range of from 21 to 28.5° C., the average being about 24° C. This room, a small inside storage room in the basement of Home Economics building, was used to store the other canned products. The third series was stored at 37° C. and the fourth at 55° C.

The temperature at which the canned beans were stored had a decided effect on the keeping qualities. No diffrence was observed between the basement and room temperatures, since there was no spoilage at either range in the 2½ or the 2 hour process. However, at 37° C. and 55° C. the amount of spoilage was 66 and 100%, respectively. This indicates that sterility was not obtained, although the process time was sufficient to yield keeping products when they were stored at lower temperatures.

Effect of character of caps and rubbers.—In this series 24 pints of beans were canned with new aluminum caps, 24 pints with new zinc caps,

24 pints with caps from jars of beans which had spoiled in 1926 and 12 pints with rubbers that had been used in 1926, but were not from jars that had spoiled. The caps from the spoiled beans had been washed when the beans were opened and had been kept at room temperature. They were washed again and boiled five minutes before using.

The beans that were canned using aluminum caps instead of zinc kept equally well. Re-use of rubbers that had been used in 1926 made no difference in securing a perfect seal or in the keeping of the jars thus treated. Using caps that had been taken from jars of spoiled beans in 1926 made a distinct difference in the keeping of the product. Thus, whereas jars processed with new caps for 2½ and 2 hours showed no spoilage, those in which the old caps were employed gave 16.6% and 25% spoilage for the respective process periods.

Effect of source of beans.—The beans in 1927 were secured from two gardens ("B" and "H"). On one day the beans were secured from both gardens to get a comparison of the spoilage. The comparison involved another factor, however, as the beans from "B" were more mature than the beans from "H". This difference in maturity was observed with the other beans from these sources.

The spoilage results were slightly different with the beans from the two gardens, although not as different as the results on the day when this was employed as the variable would indicate. Considering all of the 2 hour process beans (1927) canned by comparable methods, 61 pints were from beans secured from garden "B" and 61 from garden "H". Of these there was 6.3% and 1.6% spoilage, respectively. On the day "source" was used as a variable there was a higher percentage of spoilage than usual in the beans secured from both gardens.

Effect of consistency of pack.—In the series with variation of pack, 212 grams of beans per pint were used in the loose pack and 425 grams in the solid pack. The loose pack required more water to fill the jars after packing, while the solid pack was so tight that there was little room for water.

The consistency of the pack made no difference in the keeping qualities of the beans canned. Heat penetration figures obtained by Redfield (1927) showed a difference of at least 30 minutes in the time required for the loose and solid pack to reach the process temperature. From these figures it would be expected that at some process time lengths there would be a difference in keeping qualities, but a short enough time was not used to show this in these experiments.

Effect of addition of acid.—The beans that were canned with the addition of acid were treated and processed according to the control method except that 1.2 c.c. of 5 normal phosphoric acid or 0.8 c.c. of 5 normal citric acid were added to the cup of water used in precooking the contents of each jar. The reaction of the acidified water was pH 4.0 with the citric and pH 2.6 with the phosphoric acid. After processing, the reaction of the liquid on the non-acidified beans was pH 6.0, while that with citric or phosphoric acid was pH 5.3. After five months storage the reaction of the liquid in the non-acidified beans was pH 5.4, while that in the acidified was pH 5.3-5.4.

The addition of acid decreased the time necessary for processing the beans. There was no spoilage in the 1½ hour process when acid was

added, but on this day there was only 9.1% spoilage in the 1½ hour "no-acid" beans. In the 1 hour process, there was 45.5% spoilage in the "no-acid" beans, none in those to which citric acid had been added, and 9.1% in those to which phosphoric acid had been added. One and one-half hours was a satisfactory process time for the beans canned with the addition of acid.

Effect of storage before canning.—During 1926, two series of experiments were run to determine the influence of the freshness of the product on the keeping qualities. Enough beans for 240 pints were secured at one time. Sixty pints were canned soon after they were gathered. The beans that were kept for one, three and five days before canning were stored in market baskets in a basement room with a temperature range of from 22 to 26° C. The beans that had stood one day required ⅛ cup more water to fill the jars than the fresh beans. They appeared to be in good condition. The three day old beans were in very poor condition, some molded, some with decomposing areas, some dried and shrivelled, and the color was changed from a deep green to a yellowish green. They were tough and hard to snap. They required ¼ cup more water to fill the jars than the fresh beans. The five day old beans were in much the same condition as the three day, but showed more decomposition and drying.

The beans which were canned when fresh and processed 2½ hours or longer showed little spoilage. The beans that had stood one day or longer before they were canned showed considerable spoilage although the percentage did not increase in any regular order. The relation of the freshness of the product, process time and keeping qualities can be seen from this table.

TABLE V. Showing relation of freshness of beans to keeping qualities.

	Canned fresh	Canned after 1, 3, 5 days
Processed 2½ hours or more	1.3%*	37.5%
Processed 2 hours or less	23.4%	41.3%

*Percentages represent spoilage.

Effect of time elapsing before completion of seal.—Twenty-eight pints of beans were canned with a view to noting the effect of a slight delay in completing the seal after the beans came from the boiler. Occasionally the last jars removed from the boiler are uncovered for a short time by the water level going below the rubbers as other jars are lifted out. This experiment was carried out to see whether those that have the seal uncompleted for a time have as good suction and as good keeping qualities. With 4 jars the seal was completed in 15 seconds after removing from the boiling water, with 4 in 30 seconds, with 4 in 45 seconds and with 16 in 60 seconds.

The lapse of one minute after the jar is taken from the boiler before the completion of the seal was not enough to make any difference in the suction or keeping of the product.

Summary of beans canned under ordinary conditions.—Of the 238 pints of beans canned under ordinary conditions and processed for 2½ hours or more, there were only two pints which spoiled (0.8%). Of 144 pints which were processed for 2½ hours only 0.6% spoiled. With lower periods of heating the amount of spoilage was very much higher: the 2, 1½ and 1 hour process periods showed spoilage to the extent of 6.9%, 34.4% and

45.5%, respectively. For the beans canned under ordinary conditions, 2½ hours was a satisfactory time for processing.

The spoilage data from 906 pints of string beans canned under different conditions indicate that string beans can be processed in the hot water bath without having a high percentage of spoilage if the storage temperatures are not high, if the beans are canned soon after gathering, and if reasonable precautions are taken against adding large numbers of bacteria to the jars.

SWEET CORN

The results of the observations on sweet corn canning are summarized in Tables V and VA.

The corn was cut in the Maine style, the tips of the kernels being cut from the cob and the milk scraped out. The corn for each jar (320 grams) was mixed with 160 grams of boiling water and brought slowly to boiling. It was packed into the jar as hot as possible and processed at once. The jars were stored and observed for a period of about eight months for the 1926 series and five months for the 1927.

Effect of storage before canning.—In 1926, two series were run where freshness of the product was a variable. The corn for three days canning was brought in at one time. One portion was canned immediately, one portion was canned after one day and one after two days. It was kept in bushel baskets in a basement room at about 23 to 25° C. The corn after one and two days storage had heated, and when canned the temperature inside the ears was from 28 to 35° C. It smelled slightly sour as it was cut from the cob. After the corn was canned the reaction of that canned after storage was 0.2 to 0.4 of 1 pH unit lower than that of the fresh corn. The bacterial count of the corn increased greatly on standing, as shown below:

	Bacterial Count (Before precooking	After precooking)
A Series Fresh corn	54,000 per c.c.	50 per c.c.
1 day	825,000 " "	2 " "
2 day	1,375,000 " "	240 " "
B Series Fresh corn	31,000 " "	10 " "
1 day	230,000 " "	6 " "
2 day	190,000 " "	2100 " "

In 1927 one series consisted of corn that had been gathered the afternoon before and stored over night in the basement room at 23° C. as compared with a control series which consisted of corn from the same garden gathered by 9 o'clock the next morning and the processing begun within three and a half hours. The temperature of the freshly gathered ears of corn was about 18° C., while that of those stored overnight was between 28 and 30° C. There was no change in the pH of the canning mixture discernable by the colorometric method. The bacterial count was increased by the storage, as indicated below.

	Bacterial count (Before precooking	After precooking)
Fresh corn	3,000 per c.c.	5
Stored corn	640,000 per c.c.	20

In the three series designed to ascertain the effect of storage before canning the results obtained varied. In series "A" 1926, there was three or four times as much spoilage with the fresh corn as with that which had stood one day before canning. In series "B" the opposite was true. The fresh corn kept much better than the corn that had been stored one day. In 1927, the series "C" that was run with corn stored over night before canning showed more spoilage with the corn gathered in the morning than with that gathered the night before. Some of these results are contrary to what would ordinarily be expected. There are many factors involved. The pH change was similar in the "A" and "B" series, so would not have been the controlling factor. It seems that a possible explanation may be that in some cases the bacteria present are in more resistant form than in others. In series "A" it is possible that the bacteria, even though greatly increased in number after one day, were of a less resistant form at the time of processing. Thus the fresh material showed a bacterial count of 54,000 per c.c., which dropped on precooking to 50 per c.c., whereas the material which had been stored for one day had an initial count of 825,000, which was reduced to 2 per c.c. through the simple process of precooking. This would indicate that although there were more organisms present in the "one day" material there were actually fewer heat resistant or spore forms. After two days storage the bacterial count rose to 1,375,000, which dropped to 240 per c.c. as a result of precooking. On the basis of the relative incidence of resistant forms as indicated by the count after precooking the amount of spoilage would be expected to be greatest in the "two day" material and least in the "one day". This was in line with the results obtained. This line of reasoning is not adequate to explain all of the results, however, for in the "B" and "C" series the amount of spoilage was not correlated with the number of organisms remaining after precooking. We must assume, therefore, that the resistance of the organisms surviving the precooking process, as well as the numbers, is a determining factor.

On the basis of the results obtained, one could not predict in advance whether storage would have a favorable or unfavorable effect on the keeping qualities of corn. There would be no way of determining, previous to canning, whether or not the bacteria were in the most resistant stage, so until further work is done along this line the assumption that fresh material keeps better is the better one to follow.

Effect of delay in processing after precooking.—In this series the corn was precooked and packed into jars. Twenty-four jars were processed immediately, 24 were allowed to stand 3 hours, 24 were allowed to stand 6 hours and 24 were allowed to stand 15 hours before processing. Cooling and reheating temperature curves were noted. In one hour the precooked corn had cooled to 54° C., in two hours it had cooled to 44° C. and in three hours to 32° C. The room temperature was 29° C. It took 95 minutes for this cooled corn to reach the processing temperature after it was placed in boiling water while the corn packed hot and processed at once reached the processing temperature in 40 minutes. The bacterial count before precooking showed 7800 per c.c. of liquid, while afterward it showed 4 per c.c. The count in the same jar changed to 10 at the end of 3 hours standing, 165 at the end of 6 hours, and 22,800 at the end of 15 hours. The liquid from the 15 hour sample when boiled showed only 3 per c.c.

TABLE V. Showing spoilage records of 578 pints sweet corn canned under different conditions.

Process time in hours	4	3½	3	2	1½
Variable observed	Percentage spoiled				
Effect of storage before canning					
Series "A" (1926)					
Canned fresh	36.4	36.4	54.5	100*	
Canned after 1 day	9.1	9.1	9.1	63.6	
Canned after 2 days	81.8	72.7	80*	100	
Series "B" (1926)					
Canned fresh	9.1	0	0	72.7	
Canned after 1 day	40.0*	27.3	27.3	72.7	
Canned after 2 days	9.1	36.4	45.5	45.5	
Series "C" (1927)					
Canned fresh	18.2		9.1	63.6	
Canned after overnight storage	0		18.2	27.3	
Effect of addition of acid (1927)					
No acid added			100	100	81.8
Phosphoric acid added			0	0	18.2
Citric acid added			0	0	9.1
Effect of delay in processing after precooking (1927)					
Processed immediately			0	27.3	
Processed after 3 hours			9.1	0	
Processed after 6 hours			18.2	36.4	
Processed after 15 hours			18.2	63.6	
Effect of batch of material					
†Batch canned on 8/23, 1926	36.4	36.4	54.5	100*	
† " " " 8/26, 1926	9.1	0	0	72.7	
" " " 8/25, 1927	9.1		18.2	18.2	
† " " " 8/26, 1927	18.2		9.1	63.6	
† " " " 9/6, 1927			0	27.3	
" " " 9/9, 1927	0**		9.1	36.4	
† " " " 9/12, 1927			100	100	81.8

*Percentage based on 10 jars.

**Percentage based on 9 jars.

Other percentages based on 11 jars.

†Noted also in sections above.

TABLE VA. Summary of spoilage of sweet corn canned with batch of material and process time the variables.

Process time in hours	4	3½	3	2	1½
Number jars stored	53	22	77	76	11
Number jars spoiled	8	4	21	45	9
% spoiled	15.1	18.2	27.3	59.2	81.8

The effect of delay in processing after precooking is uncertain. A delay of three hours gave one jar more spoilage in the three hour process, but considerably less in the two hour. A delay of six or fifteen hours resulted in more spoilage in both the three hour and the two hour process.

Effect of addition of acid.—In this series 2.6 c.c. 5 normal phosphoric or 2.0 c.c. 5 normal citric acid was added to 160 grams of cold water poured over the 320 grams of corn for each jar before precooking. The reaction of the water containing phosphoric acid was pH 2.4 and that containing citric

was pH 2.9. The acidified corn after processing was pH 6.0, while that with no acid added was pH 6.9.

The addition of acid had a pronounced beneficial effect on the keeping of the sweet corn. On the day the addition of acid was used as a variable, there was almost 100% spoilage in that canned without acid, while with the acid there was no spoilage in the 2 or the 3 hour process and comparatively little in the 1½ hour. The sweet corn canned with acid had an acid flavor that was strong enough to be objectionable to some individuals. However, when ¼ teaspoon of soda was added to the pint of corn, as it was heated for serving, the acid flavor disappeared and a palatable product resulted. Living organisms which grew and produced acid but not gas in dextrose broth tubes were obtained from 29 of the 33 jars canned without acid on this day, from 4 of the 33 jars canned with phosphoric acid and from 2 of the 33 jars canned using citric acid.

The two hour process time with the addition of either citric or phosphoric acid gave satisfactory results.

Effect of batch of material.—Corn canned on different days showed considerable variation with respect to the proportion which spoiled. Thus of eleven jars prepared on each of a number of days and processed for 3 hours the spoilage records were as follows: Aug. 26, 1927—none; Sept. 6, 1927—none; Aug. 26, 1927—one; Sept. 9, 1927—one; Aug. 26, 1927—two; Aug. 23, 1926—six; Sept. 12, 1927—all. This made a variation of from 0 to 100% in the amount of spoilage using the same method and process time on different days.

The variation in keeping qualities on different days for the various process periods may be summarized as follows:

2 hour process—18 to 100 % spoilage
3 hour process— 0 to 100 % spoilage
3½ hour process— 0 to 36.4% spoilage
4 hour process— 0 to 27.3% spoilage

These results with corn treated as nearly the same as possible showed that an undetermined factor influenced greatly the keeping of the corn. No explanation of the difference in keeping could be gained from a comparison of the maturity of the corn and the resulting difference in consistency of pack and heat penetration, the previous weather conditions, the bacterial count before and after precooking, the pH of the corn, the pH of the water used, the titrable acidity of the corn, the temperature of the ear of corn, or the barometric pressure on the days of canning. Probably the controlling factor is the nature and heat resistance of the organisms that happen to be present on the corn at the time of canning.

Since the difference in spoilage is due to a factor which we did not control and have not definitely determined, we are unable to give data for a completely satisfactory process time for corn without the addition of acid. When the acid was added to the extent of 2.0 to 2.6 c.c. of 5 normal citric or phosphoric acid for each pint of corn, there was no spoilage after two hours processing in the water bath.

SWISS CHARD

Methods used in canning.—The general method used for canning vegetables was used for the chard except that the pans used to precook the chard were large, tightly covered sauce pans. In 1926, only two table-

spoons of water were added to each 475 grams of chard in the precooking. In 1927, ¾ cup of water and 350 grams of chard were used for each pint jar. In 1926, the chard was opened after seven months and in 1927 after about five months storage.

The chard that was allowed to stand one day before canning was kept at a temperature of 24° C. in bushel baskets. It was not wilted enough to be noticeable, but had lost 5.5% in weight during storage.

The chard canned with a loose pack contained 204 grams per pint, while that with a solid pack contained 475 grams per pint. Aluminum caps were used on 12 additional pints canned with the loosely packed chard.

The jars canned to show the effect of delay in processing after precooking were allowed to stand in a warm room (24° C). during the interval between packing and processing. Bacterial counts at intervals, and temperature cooling and re-heating curves were taken. The number of bacteria in the chard after precooking did not increase appreciably in three hours and was only doubled in five hours storage. The chard had cooled to 56° C. at the end of one hour, to 42° C. at the end of two hours and to 36° C. after three hours standing in a room at a temperature of 24° C. It took 45 minutes for the jar that had cooled to 36° C. to reach the processing temperature after it was put into the hot water bath, while the chard which was processed immediately after precooking reached the processing temperature in 15 minutes. This difference was due to the difference in initial temperature in a product with slow heat penetration.

The chard that was canned with the addition of acid contained 1.5 c.c. of 5 normal phosphoric acid or 1.0 c.c. of 5 normal citric acid per 190 c.c. water which was added to each jar. The reaction of the liquid used was pH 3.6 for the citric and pH 3.2 for the phosphoric acid. After processing the reaction of the liquid on the non-acidified chard was pH 6.1, that treated with phosphoric acid was pH 5.8 and that to which citric acid had been added was pH 5.6. After five months' storage the reaction of all jars of chard was about pH 5.4.

Results.—The results obtained from the Swiss chard canning are summarized in Tables VI, VII and VIIA.

Swiss chard canned fresh kept much better than that allowed to stand one day after gathering before canning. This is shown in Table VI.

TABLE VI. Showing the relation of storage before canning to spoilage.

	Canned fresh	Canned after 1 day storage
Processed 2½ hours or more	2.3%*	13.6%
Processed 2 hours	27.3%	45.5%

*Percentages represent spoilage.

In the series of experiments carried out to ascertain the effect of the consistency of the pack, the Swiss chard packed loosely kept better than that packed solidly. There was no spoilage in either pack with the 2½ hour process. For the 2 hour process there was no spoilage in the loosely packed, but 18.2% in the solidly packed jars. For the 1½ hour process the spoilage was 9.1 and 36.4% for the loose and solid packs, respectively. Heat penetration is slow in a substance with the consistency of Swiss chard and the more solid the pack the longer time required for the center of the

jar to reach the processing temperature. This difference in rate of heat penetration into the center of the jar accounts for the greater spoilage in the firmer pack.

The chard canned with aluminum caps kept as well as that for which zinc caps were used.

The chard that was processed at once after precooking showed considerably less spoilage than that in which there was a three or five hour delay in processing. In view of the fact that this delay resulted in a marked drop in temperature (from 87° to 36° C.), the actual period this material which had stood was subjected to the processing temperature was about thirty minutes less than that of those jars which were processed immediately after precooking. This was shown by the heat penetration curves previously mentioned.

TABLE VII. Showing spoilage records of 352 pints of Swiss chard canned under different conditions.

Process time in hours	4	3½	3	2½	2	1½	1
Variable observed	Percentage spoiled						
Effect of storage before canning (1926)							
Chard canned fresh	0	9.1	0	0*	27.3		
Canned after 1 day storage	9.1	18.2	18.2	9.1	45.5		
Effect of consistency of pack (1927)							
Loose pack				†0	0	9.1	
Solid pack				0	18.2	36.4	
Effect of delay in processing after precooking (1927)							
Processed immediately				0		9.1	
Processed after 3 hours				0		54.5	
Processed after 6 hours				18.2		54.5	
Effect of addition of acid (1927)							
No acid added					9.1	18.2	63.6
Phosphoric acid added					0	0	9.1
Citric acid added					9.1	0	0

*Percentage based on 10 jars.

†Percentage based on 23 jars (12 with aluminum caps).

Other percentages are based on 11 jars.

TABLE VIIA. Summary of spoilage of jars of Swiss chard canned under ordinary conditions.*

Process time in hours	4	3½	3	2½	2	1½	1
Number jars stored	11	11	11	55	44	44	11
Number jars spoiled	0	1	0	0	6	8	7
% spoiled	0	9.1	0	0	13.6	18.2	63.6

*Excludes material stored before or after precooking and material to which acid was added.

The addition of acid to the chard increased the probability of its keeping at the shorter process time lengths. In the 1 hour process, 63.6% of the non-acidified chard spoiled, while 9.1% of that with phosphoric acid spoiled and none of that with citric acid. In the 1½ hour process, 18.2% of the "no acid" spoiled and none of that to which either citric or phos-

phoric acid had been added. In the 2 hour process, 9.1% of the "no acid", none of that to which phosphoric acid was added and 9.1% to which citric acid was added spoiled. This 9.1% represented only 1 jar and the absence of spoilage in the 1½ and 1 hour processes with the citric acid series would indicate that this is a chance occurrence and ordinarily we should anticipate no spoilage with the 2 hour process in the presence of the acid.

Of the 55 jars of Swiss chard canned under ordinary conditions, there was no spoilage in the 2½ hour process. The fact that one of the jars processed 3½ hours spoiled makes a spoilage percentage of 1.1 for the 88 jars processed 2½ hours or more. The percentage of spoilage at other process time lengths was 13.6% for the 2 hour, 18.2% for the 1½ hour and 63.6% for the 1 hour. Two and one-half hours was a satisfactory process time for the chard canned.

TOMATOES

Methods used in canning tomatoes.—The tomatoes secured for canning were in poor condition due to a rainy season. When the tomatoes were brought to the laboratory they were sorted and all that showed decomposition were discarded. Thus on the first day only those which showed no decomposing areas were used. Tomatoes to be canned two days later were stored in bushel baskets at a temperature of 21-22° C. After storage, many of the tomatoes had rotted places in them. These were cut out and the tomatoes used. The tomatoes were washed, put into wire baskets, immersed in boiling water for 30 seconds, dipped in cold water and taken out at once. The skins were removed. The tomatoes were cut into quarters and packed tightly into hot jars, and the jars filled to within ¼ inch of the top with tomato juice. The jars were left in hot water (50° C.) for one minute to prevent breakage when plunged into boiling water. The tomatoes canned fresh had a bacterial count of about 500,000 per c.c. of juice as they went into the jar, while those canned after two days storage had a count of over 2,500,000 per c.c.

The storage period for the processed tomatoes was between seven and eight months.

Results.—The condition of the tomatoes when canned made a decided difference in their keeping qualities, as shown in Tables VIII and IX. Tomatoes canned on the day they were gathered showed no spoilage when processed 20 minutes or longer, while those stored for two days before canning showed spoilage in all processes under 35 minutes.

TABLE VIII. Showing the relation of storage before canning to spoilage.

	Canned fresh	Canned after 2 days
Processed 20 minutes or more	0 %*	14.6%
Processed 12 minutes or less	52.4%	42.9%

*Percentages represent spoilage.

The relation of the keeping of the tomatoes to the temperature reached in the center of the jar is shown by the heat penetration figures on tomatoes obtained by Redfield (1927) and noted below.

Length of time of process (in minutes)	Maximum Temperature reached in center of jar—°C.	Number of minutes center of jar was above 60° C.*	Number of minutes center of jar was above 70° C.
5	48.6	0	0
12	57.2	0	0
20	71.1	>35	4
25	72.7	>40	18
35	84.1	>40	33
45	90.5	>55	41

*Taken as an arbitrary temperature for evaluating killing time of non-sporeformers.

It is interesting to note that with the fresh product sterilization was adequate when the process was long enough for the center of the jar to reach 71° C. Because the center temperature continues to rise after the jars are removed from the boiler, the 20 minute process kept the contents at the interior of the jar above 60° C for over 35 minutes.

Twenty minutes was an adequate process time for tomatoes canned shortly after they were gathered, but inadequate for tomatoes that had been kept two days and showed evidence of rot before canning.

TABLE IX. Showing effect of storage of tomatoes before canning on the length of Time Necessary to Process (126 pints).

Process time in minutes	45	35	25	20	12	7
Variable observed	Number spoiled					
Tomatoes canned fresh	0	0*	0	0	6	5*
Tomatoes canned after 2 days	0*	0*	2	4*	1	8*
	Percentage spoiled					
Canned fresh	0	0*	0	0	54.5	50*
Canned after 2 days	0*	0*	18.2	40*	9.1	80*

*These figures represent spoilage from 10 jars.
Other figures represent spoilage from 11 jars.

BEEF

Methods used in canning beef.—The beef canned was round and forequarter cuts from 20 month heifers which had been killed and kept for one to two days at 0 to 2° C. before processing. Beef was secured from two lots of animals having a similar history, a control series being run each time. The meat was secured from the meat laboratory of the Animal Husbandry Department of the college.

For the control method the meat was wiped with a damp cloth, cut into pieces convenient for packing and 453 gram portions were packed into hot jars. One teaspoon salt was used to each pint jar. The filled jars were placed in hot water for one minute to avoid breakage when plunged into the boiling water.

In one series the relative quantity of water present was used as a variable. Seventy-five c.c. boiling water was added to fill the interspaces in the jars.

To ascertain the effect of the addition of bone in canning beef, a small part of the round bone was allotted to each jar and the weight made up to 453 grams with lean beef.

In another series of observations the effect of the relative amount of fat was studied. This was done by replacing 100 grams of the lean beef by suet in 48 of the jars.

In another series of 48 jars, the beef was partially cooked before being put into the jars. It was weighed, seared in hot frying pans until well browned and packed hot into the hot jars. Seventy-five c.c. of hot water was put into each frying pan and heated to boiling and this unthickened brown sauce was poured over the meat.

Another series was made up of beef that had been allowed to ripen at temperatures from 0-2° C. for 15 days before canning.

The finished jars of beef were stored at about 24° C., observed from time to time and those jars showing evidence of spoilage were removed and examined chemically and bactriologically. The total period of observation was eight months. At the end of this time the remaining jars were examined.

Results.—No spoilage was shown by the 151 pints of beef processed for 3 or 4 hours. There was 2.6% spoilage in the beef processed 2 hours and 100% of the beef that was processed for 1 hour spoiled. The variables, the presence of bone, the relative amounts of water and of fat, precooking and ripening before canning, made no significant difference in the keeping qualities of the beef. The results obtained with beef are shown in Table X. Three hours was a satisfactory process time for the beef canned under the conditions of the experiments.

TABLE X. Showing spoilage record of 301 pints of beef canned under different conditions.

Process time in hours	4	3	2	1
Material used	Percentage spoiled			
Control beef "A"	0	0	0	100†
Beef with water added	0	0	0	100
Beef with bone added	0	0*	0	100
Beef with fat added	0	0*	10*	100
Control beef "B"	0	0*	0	100*
Beef browned before packing	0	0	8.3**	100
Beef ripened before packing	0	0	0	100*
Summary				
Total jars stored	77	74	77	73
Total jars spoiled	0	0	2	73
Total % spoiled	0	0	2.6	100

*10 jars stored.

**12 jars stored.

†9 jars stored.

Other percentages based on 11 jars stored.

PORK

Methods used.—The variables employed in the canning of pork aside from length of process time were the addition of water, addition of extra fat, precooking before processing and the use of ground loin as compared

with cut pieces of loin. The control method was that used for the beef, 453 grams loin being used for each jar. The loin from 10 months old hogs was used. This was procured one to five days after the hogs had been killed. Loin was secured from two similar lots of hogs, a control series being run each time.

When water was added as a variable 45 c.c. was enough to fill the interspaces of the jar.

The ground loin (sausage) procured was made up of about 70% lean and 30% fat. The amount of sausage used in each jar was 475 grams. The sausage with extra fat added consisted of 425 grams sausage and 50 grams added fat. This made about 37% fat in the sausage.

For the sausage that was partially cooked before packing, 475 grams were weighed and formed into cakes (seven cakes per jar). They were browned well in a frying pan and packed hot into the jars. The fat from the pan was poured over the sausage.

The storage period for the canned pork was over eight months.

Results.—Of the 130 pints of pork processed 3 or 4 hours none showed any evidence of spoilage. These results are summarized in Table XI. Of those given the 2 hour process, 2 of the 10 jars of sausage with extra fat added spoiled. No other spoilage occurred in the 2 hour process. In those jars processed one hour, spoilage was very high, from 45.5 to 100% in all except the sausage that had been cooked before packing. In this series there was no spoilage.

The only variable, aside from time of process, which made a marked difference in the keeping of the product, was cooking of the sausage before packing.

The spoilage in the 2 hour process in the sausage with extra fat added may or may not have been accidental. The heat penetration figures of Redfield (1927) with packs of sausage similar to these showed the sausage with extra fat added took 20 minutes longer to reach the processing temperature than the sausage without the extra fat. This may have been a reason for more spoilage in the series with extra fat. Three hours was a satisfactory process time for the pork canned.

TABLE XI. Showing spoilage records of 262 pints of pork canned under different conditions.

Process time in hours	4	3	2	1
Material used	Percentage spoiled			
Control loin	0	0	0	45.5
Loin with water added	0	0	0	54.5
Control sausage "A"	0*	0	0	100
Sausage with extra fat	0	0	20*	100
Control sausage "B"	0	0	0	100
Sausage browned before packing	0*	0	0	0†
Summary				
Total jars stored	64	66	65	67
Total jars spoiled	0	0	2	44
Total % spoiled	0	0	3.1	65.7

*10 jars stored.

†12 jars stored.

Other persentages based on 11 jars stored.

SUMMARY

1. Spoilage records on 2732 pints of vegetables and meats canned by the hot water bath method are presented.

2. The main variable used throughout the experiments was the length of process time. Other variables for vegetables were, various periods of storage before canning, precooking, scalding or packing cold, addition of phosphoric and citric acids, delay in processing after precooking, character of caps and rubbers, consistency of the pack, lapse of time before completion of the seal, and different storage temperatures after canning. Variables for meats other than process period were the addition of water, of bone, and of fat, partially cooking the meat before processing, the use of ripened beef and the use of ground pork.

3. Process times found entirely satisfactory, under the conditions of the experiments, were 2½ hours for beans and chard, 3 hours for beef and pork, and 20 minutes for tomatoes.

4. A process period of 3 hours for asparagus and 4 hours for sweet corn did not give satisfactory results. When these products were acidified with either phosphoric or citric acid, a process period of 2 hours was found to be sufficient for preservation.

5. Aside from the period of processing, the factors of greatest significance as regards vegetables were length of storage before canning, addition of acid, storage temperature after canning, previous history of the caps employed, consistency of the pack (with chard) and delay in processing after precooking.

6. Precooking the sausage improved the keeping of the product. Other variables studied with meats, aside from processing period, showed no significant differences.

7. Whether better results would be obtained by the home use of the pressure cooker than by using the hot water bath method for the periods found to be satisfactory in this study remains to be established.

8. The work reported in this paper was concerned with the keeping qualities of the processed materials, but the question of the possibility of food poisoning, if the material has been infected with such organisms as *Cl. botulinum,* was not investigated. We are not aware of any evidence, however, of botulinus poisoning from foods canned in Iowa.

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THE INHERITANCE OF RESISTANCE TO THE DANYSZ BACILLUS IN THE RAT[1]

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During recent years, an increasing amount of experimental evidence has added support to the belief that heredity plays an important role in the resistance of the host to an infecting organism. Tyzzer (6) and Hagedoorn (2) have each reported distinct hereditary differences in resistance to natural epidemics among their laboratory mouse stocks. Frateur (1) proposed a single factor pair as governing resistance and susceptibility to avian diphtheria; Roberts and Card (5), and Lambert (3) show results indicating that stocks of poultry more resistant to bacillary white diarrhea and to fowl typhoid, respectively, may be built up by selective breeding, while Webster (7) has demonstrated the possibility of producing stocks of mice more resistant to mouse typhoid infection than a random group of mice not so selected. Pritchett (4) asserts clear-cut differences in susceptibility to mouse typhoid between inbred strains of mice. Wright and Lewis (9) found marked differences in resistance to tuberculosis among inbred families of guinea-pigs.

The experiment of which this paper is a preliminary report was planned to study the influence of heredity on resistance to a bacterial disease by mating survivors of a fixed dose of a specific organism; in short, to produce a highly resistant strain and a highly susceptible strain of rats to a standard dose of the organism.

MATERIALS AND METHODS

The animals used in this experiment came from two distinct sources. The random stock rats (Ra) are descended from two pairs obtained in October, 1925, from Prof. V. E. Nelson of the Chemisty Department, pen inbred by him for six years. In our laboratory, they have been inbred, but not brother-sister. A trio of each of the Wistar "A" and "B" strains was obtained from Dr. Helen Dean King in October, 1924. They have since been maintained by brother-sister matings.

The housing, care, and diet of the animals used in the experiment have been kept as uniform as possible. The age at injection has been approximately 50 days. Naturally, the weight of the animals at this age will vary somewhat, depending in large part upon the number in a litter. Any differences in resistance due to differences in weight will be dealt with in a future report.

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A culture of the Danysz bacillus was obtained in October, 1926, from Dr. R. W. Spray of West Virginia University. This organism had been isolated by him from a commercial rat virus and cultured on plain agar slants. Since arrival at our laboratory, it has been carried on by monthly cultures on veal infusion agar slants.

The pathogenicity of the organism was established at the first injection; after which the procedure outlined below has been followed throughout the experiment.

The media and incubation period of the bacterial culture have been kept constant. Each 18 hr. culture of the Danysz bacillus was washed off in 4 c.c. of sterile physiological salt solution, the suspensions from the different tubes used were mixed in a serum bottle. Since the organism is motile, to facilitate counting it was necessary to heat a 0.5 c.c. sample of the total suspension at 55-60° C. for 20 minutes. Counts were made from the attenuated sample on a Max Levy counting chamber, Helber slide. After the number of organisms per c.c. was determined, the original suspension was diluted to give the desired number of organisms per c.c. Each animal was injected intraperitoneally with 0.25 c.c. of the determined suspension. The deaths for the preceding 24 hours were recorded each morning at about 10 o'clock. In general, only those deaths were recorded which occurred between the 2nd and 14th days.

Since considerable time elapses after making the suspension of bacterial cultures in physiological salt solution until counting and injecting are completed, the number of organisms injected may vary somewhat from that determined in counting. Tests are in progress to determine the viability of the Danysz bacillus in saline solution. Winslow and Brooks (8) report marked differences in viability between different types of bacteria in suspension in distilled water and likewise in saline solution. However, the time between making the suspension, counting the organisms, and injecting the animals has been relatively constant throughout the experiment. Any change, then, in the number of organisms in the suspension will be in a constant relative ratio to the number injected.

The virulence of the organism has remained practically constant, judging from the results obtained by injecting the same number of organisms into control stocks from month to month. The writer has noticed no results that would indicate a change in the virulence since the beginning of the experiment.

Determination of the Standard Dose

As a preliminary step, it was deemed necessary to determine the effect of different numbers of organisms upon the host. A dose sufficient to cause the death of approximately 85% of the animals tested was the goal desired. This number, when determined, was to be used as a minimum for further work, and has been termed the "standard dose", as opposed to the "minimum lethal dose". The results of these preliminary trials to fix the standard dose are shown in Table I.

The results of these preliminary tests with different numbers of the Danysz bacillus indicate a close correlation between decrease in dose and decrease in mortality. As the number of bacteria increases, the effect as shown by the mortality becomes less; seemingly, then, individuals resistant to a relatively large number of organisms are affected only slightly by a further increase in the number of bacilli injected.

TABLE I. Percentage mortality in rats due to varying doses of the Danysz bacillus.

Dose (Millions)	Number Tested	Number Dead	Percentage Mortality
4000 +	70	69	98.5
2-4000	39	39	100.0
275	104	98	94.2
210	93	85	91.3
150	168	139	82.7
120	72	46	63.9

A mathematical analysis of the differences in percentage of mortality due to successive, varying numbers of organisms gives no certainty that factors other than random sampling are the cause. The table, however, is a summary of tests over a period of a few months, and gives the average for the tests of each dose. It may be added that the range for the percentage mortality of each dose was quite narrow. For example, the dose of 150×10^6 organisms produced an average percentage mortality of 82.7. The range for all percentage mortalities produced by this dose is from 77.5 to 86.3; one-half the results lie within the limits of 80.4 to 85.0.

This number of organisms (150×10^6) was chosen as the standard dose and has since been used as the minimum number of organisms to be injected.

Effect of Selection

TABLE II. Percentage mortalities of stocks of rats following injection of the standard dose of the Danysz bacillus.

Stocks	Number Injected	Number Dead	Percentage Mortality
Wistar "A" strain	96	30	93.7
Wistar "B" strain	31	25	80.6
Random Stock (Ra)	168	139	82.7
R_1 stock	123	48	39.0
R_2 stock	71	17	23.9
R_1A stock	147	69	46.9

In the above table, the nomenclature of the different stocks is as follows: the Wistar "A" and "B" strains, and the random stock (Ra), are our regular laboratory stock rats. The progenies of Ra survivors of the standard dose of the Danysz bacillus represent the first resistant generation, R_1; likewise R_2 denotes the second resistant generation. Individuals of the R_1 stock surviving the standard dose were mated to non-tested "A" strain males and females; the progeny are termed R_1A stock. Until October, 1927, the Ra stock has been used as control stock in the experiment; since that date, the "A" strain has served as controls at the different injections.

It will be noted in Table II that the Wistar "A" strain was somewhat more susceptible to the standard dose than the Wistar "B" strain. However, the probability ($P = .64$) that this difference was due to other than random sampling is slight, although there are other factors which would seem to indicate that something other than chance has determined the difference. A uniformly higher percentage of deaths of the "A" strain which has occurred in a series of injections tends to strengthen the

TABLE III. Mathematical analysis[1] of differences in percentage mortality between stocks of rats shown in Table II.

Stocks compared	Differences in Percentage Mortality	X^2	P
"A" and "B"	13.1	0.24	0.64
R_1 and Ra	43.7	13.64	*
R_1 and R_2	15.1	2.31	0.13
R_1 and "A"	54.7	15.52	*
R_1A and "A"	46.8	11.31	*

*Probability very great that the differences are due to causes other than chance ($P<0.01$).

[1]The calculations have been made according to Fisher's "Contingency Tables". Fisher, R. A. 1925. Statistical Methods for Research Workers, p. 84. Oliver and Boyd, London.

belief that a significant difference would appear if a larger number could be used. Hence the "A" strain has been selected as a more susceptible strain for purposes which will be dealt with a little later in this paper.

There was practically no difference between the "B" strain and Ra stocks in their reaction to the standard dose. In this report, the survivors of the standard dose giving rise to the R_1 stock have been confined to the Ra stock. The first resistant generation of the inbred "B" strain (R_1 "B" strain) is being developed at present; but the results as yet are in insufficient numbers to be included in this paper.

The R_1 stock showed a decided increase over the control stock injected at the same time in the number of animals surviving injections of the standard dose. A comparison of the difference in resistance between the R_1 stock and the average of the parental Ra stock gives a value for X^2 of 13.6. Here it is certain that factors other than chance have operated in determining the difference in resistance.

Likewise, the R_2 stock in turn gave an average of more individuals resistant to the standard dose than the average of the R_1 stock. The difference between these two stocks approaches significance ($P = .13$) and may be interpreted as having been due in large part to selection within the R_1 stock.

Previous to the testing of the R_2 generation, the R_1 stock and the "A" strain differed most widely in resistance. It has been stated before that the "A" strain is considered as the "susceptible" strain. The reaction of the progeny from matings between these R_1 and "A" stocks has been of value from two distinct points of view; it has afforded an opportunity to study the method of inheritance of the resistance, and to determine the effect of any passive immunity that might have been transmitted by the survivors to their offspring.

The increase in percentage of individuals of this progeny, R_1A stock which survived the standard dose, is significant when compared with the mortality of the "susceptible" "A" strain parent. The odds are extremely great that the increase in resistance of this stock was in large part due to the influence of the R_1 parent, since X^2 for this difference equals 11.31.

The resistance was contributed to this progeny by either R_1 parent as shown below:

Parents	Number Tested	Number Dead	Percentage Mortality
R_1 ♂♂ x A ♀♀	85	38	44.7
R_1 ♀♀ x A ♂♂	62	31	50.0

It will be noted that there was an approximate equality of the R_1A stock, when the R_1 parent was either male or female. The seeming difference in mortality between these progenies (44.7-50.0) was largely due to the difference in mortality between sexes in this particular progeny. An analysis of this difference is shown as follows:

Mating	Sex	Number Tested	Percentage Mortality
R_1 ♂♂ x "A" ♀♀	♂♂	46	39.1
R_1 ♂♂ x "A" ♀♀	♀♀	39	51.2
R_1 ♀♀ x "A" ♂♂	♂♂	27	44.4
R_1 ♀♀ x "A" ♂♂	♀♀	35	54.2
Total	♂♂	73	41.0
Total	♀♀	74	52.7

There was a somewhat higher number of deaths among the females in this progeny than among the males. Since there was a larger number of females in proportion to the males in the offspring of the cross, R_1 ♀♀ x "A" ♂♂, the percentage mortality of this offspring would necessarily be increased slightly as compared with the reciprocal cross (R_1 ♂♂ x "A" ♀♀), in which the males were in a slight majority.

Were the increased resistance of the R_1 and R_2 generations over the Ra stock due in part to the transmission of passive immunity, the offspring of the mating R_1 ♀♀ x "A" ♂♂ should have shown a higher degree of resistance than those from the reciprocal cross (R_1 ♂♂ x "A" ♀♀) due to the influence of the dam. There is no case reported in the literature within the knowledge of the author in which an immunized male has transmitted passive immunity to the offspring. Hence, the effect of passive immunity as a factor in increased resistance of selected stocks in this experiment, is at a minimum, since the progeny (R_1A) from the cross, R_1 x "A", has shown no difference in effect from either parent being resistant.

SUMMARY

The number of organisms of the Danysz bacillus that was injected determined within reasonable limits the percentage mortality of a random sample of rats.

Slight differences in resistance to the standard dose of the Danysz bacillus have been found between two inbred strains of rats, Wistar "A" and "B". In a random stock of rats progeny from matings of survivors of injection of a fixed number of this organism, were more resistant to the same dose than a random group of the same stock.

Offspring of a cross between the first resistant generation and the susceptible "A" strain were much more resistant to the disease than the susceptible parent. There was no evidence that this increase in resistance was due even in part to the transmission of passive immunity.

It is suggested from these results that resistance in this case is due to a partially dominant, quite complex set of factors, whose interactions with the environmental factors determine the reaction of the individual to the infection.

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SPECIES CROSSES IN THE GENUS CUCURBITA

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INTRODUCTION

A perusal of the literature dealing with interspecific hybrids in Cucurbita leaves considerable uncertainty as to the limits of intercrossing in this genus. Certain of the investigators in this field were wholly unable to secure hybrids between the species, whereas others achieved positive results; the present work was undertaken, therefore, with the hope of throwing additional light on the problem.

Five of the ten species comprising this genus have been employed by previous investigators in their hybridization experiments. In the writer's investigations three species were used—*C. pepo, C. maxima,* and *C. moschata,* these being the only annual species in the genus, as well as the only species cultivated in the United States.

The results given herein represent the work of six consecutive years carried on in both field and greenhouse in an effort to secure hybrids among the three above mentioned species.

HISTORICAL

As early as 1854 Naudin (10) attempted to intercross various species of Cucurbita, viz: *C. maxima, C. pepo, C. moschata, C. melanosperma* and *C. perennis.* The result of a comparatively small number of controlled pollinations was a few fruits, none of which contained fertile seeds.

It was more than thirty years later that a similar line of investigation was undertaken by Bailey (1, 2). He worked for ten consecutive years cross-pollinating varieties, species and genera of the Cucurbitaceae. As a result of numerous efforts with the three cultivated species of Cucurbita, seven fruits were secured: two by pollinating *C. pepo* x *C. maxima,* one by *C. moschata* x *C. maxima,* one by *C. maxima* x *C. pepo,* and three by *C. pepo* x *C. moschata.* However, fertile seeds were found only in the three fruits obtained from *C. pepo* x *C. moschata,* and eighty-eight F_1 plants were grown from the seeds in the two fruits resulting from Connecticut Field Pumpkin x Japanese Crookneck. (No mention is made of the seeds from the third fruit—Gourd x Japanese Field Pumpkin.) A number of F_2 plants were also grown and one fruit secured, but Bailey does not state whether this fruit contained fertile seeds. Somewhat at variance with the findings of Bailey are those of Pammel (11), who concluded it is impossible to obtain hybrids between the different species of Cucurbita.

Additional data have been furnished by Drude (4, 5), whose researches extended over a period of twenty-five years. Only three fruits were produced and these by the pollination of *C. pepo* (white apple) with *C. ficifolia* (C. melanosperma). Two of these fruits were entirely sterile, the third contained one fertile seed. This hybrid was in turn successfully crossed with a Fordhook (*C. pepo*). Drude's attempts between 1901 and 1905 to repeat this cross between *C. pepo* and *C. ficifolia* failed, as did all attempts

to cross other species of the genus during his twenty-five years of experimentation.

The work of the Hagedoorns (6, 7) is of considerable interest in that they not only secured several interspecific hybrids (*C. maxima* x *C. pepo*), but also apparently found that some of the F_1 hybrid plants set fruit and produced viable seeds from unpollinated female buds. Only three F_1 interspecific and two F_1 intervariety hybrids developed parthenogenetic seeds. The F_2 and F_3 generations in each case were grown for the purpose of ascertaining whether true parthenogenesis or merely apogamy was indicated. By this genetic method it was found that in at least four of the five cases the progenies gave strong evidence of parthenogenesis. That parthenogenesis in Cucurbita is closely associated with hybridity finds support in the fact that 106 female flowers of 18 varieties, carefully protected from pollination, failed to produce a single seed. It is worthy of note that the three fruits resulting from the pollination of *C. maxima* (Turkenbund) x *C. pepo* developed on a single Turkenbund plant. Their efforts to intercross species other than *C. maxima* x *C. pepo* were unsuccessful.

In discussing the investigations carried on by the Hagedoorns, Lotsy (8, 9) thinks the possibility of crossing *C. maxima* with *C. pepo* has not been proved. Although he made unsuccessful attempts to cross these species himself, he feels his work has not been sufficiently intensive to definitely conclude that crosses cannot be made betwen *C. maxima* and *C. pepo*. He is convinced, however, from his own investigations that neither *C. pepo* nor *C. maxima* can be crossed with *C. melanosperma*. Lotsy's results do not confirm those of the Hagedoorns regarding the occurrence of parthenogenesis in unpollinated F_1 hybrids.

The most recent work with a view to crossing the species of Cucurbita is that of Vavilov (12), who made reciprocal pollinations among four species—*C. pepo, C. maxima, C. moschata* and *C. melanosperma*. Not only was he unsuccessful in obtaining hybrid seed, but in not a single case was fruit secured as a result of his pollinations. He, too, was unable to confirm the phenomenon of parthenogenesis as reported by the Hagedoorns. Vavilov cites the work of Miss Koslov (unpublished), at the Turkistan Agricultural Experiment Station, who secured four hybrid seeds by pollinating *C. maxima* with *C. moschata*. The F_1 fruits were entirely without fertile seeds, as Vavilov personally observed in December, 1924. His conclusion is: "Species of Cucurbitaceae are so different that to get fertile hybrids among them, and especially among different species of Cucurbita, is impossible."

Summarizing the results reported in the above literature, the interspecific hybrids obtained were a considerable number of fertile F_1 hybrids of *C. pepo* x *C. moschata*, by Bailey; one F_1 hybrid plant of *C. pepo* x *C. ficifolia* (C. melanosperma), by Drude; a number of F_1 hybrids of *C. maxima* x *C. pepo* by the Hagedoorns, and four sterile hybrids of *C. maxima* x *C. moschata*, by Miss Koslov (unpublished).

MATERIAL AND METHOD

The investigations were carried on with two general types of seeds—pure lines, and that obtained from numerous commercial seed houses throughout the United States. In all cases numbers have been used to designate the pure lines, while variety names have been employed for commercial forms.

The sources of pure lines are:

Line No. 175—Connecticut Field Pumpkin (*C. pepo*) developed by the writer by inbreeding.
Lines No. 48a, b, c—Patty Pan, or Scallop, Pumpkin (*C. peppo*), from Dr. E. W. Sinnott, of the Connecticut Agricultural College.
Line No. 270—Hubbard Squash (*C. maxima*) from M. B. Cummings, of the Vermont Agricultural Experiment Station.
Line No. 20—Hubbard Squash (*C. maxima*) from John W. Bushnell, then of the Minnesota Agricultural Experiment Station.
Line No. 5—Large Cheese, or Kentucky Field, Pumpkin (*C. moschata*), developed by the writer by inbreeding.

Plants were all grown on the grounds or in the greenhouse of the Iowa Agricultural Experiment Station. The work consisted of cross pollinating varieties between the three species, and since descriptions of these species are given in an earlier paper by Castetter and Erwin[3], these will not be included here. As Cucurbita is monecious and has very large flowers it was easy to prevent contamination by foreign pollen. The method used was to isolate both staminate and pistillate flower buds by tying the corolla with a heavy cord on the evening previous to opening. On the following morning the cord was removed from each flower and the stamens from the desired variety rubbed over the stigma of the pistillate flower, which was again tied with the cord, properly tagged and labelled—the various types of pollinations being designated by tags of different colors attached to the fruit stalk. Especial care was exercised in tying the buds and flowers to preclude the possibility of pollination by insects; also flowers torn in handling, or those concerning which there was any doubt as to complete isolation, were discarded. Immediately before being removed from the vines all fruits were tagged a second time to prevent confusion of identity in handling. Fruits were allowed to remain unopened for a month or more after being removed from the field or greenhouse, in order to allow seeds to fully mature.

RESULTS

Each series of pollinations, with the fruits and seeds resulting therefrom, is given in condensed tabular form according to species, and is a summary of investigations carried on in both field and greenhouse over a period of six years.

C. PEPO x *C. MAXIMA*

Parents	Number Pollinations	Fruits	Fertile Seeds
Connecticut Field x Hubbard	93	10	0
175 x 270	51	4	0
175 x 20	70	4	0
Connecticut Field x Marblehead	152	12	0
Connecticut Field x Victor	120	6	2
48a x 20	54	17	0
48b x 20	74	8	0
48c x 20	53	17	6
	667	78	8

From the above table it will be seen a number of parthenocarpic fruits were secured. In neither successful cross, however, were the fertile seeds all found in a single fruit. Of the two obtained from Connecticut Field x Victor, one seed was found in each of two fruits. The six seeds resulting from the 48c x 20 cross were distributed among four fruits. These eight hybrid seeds appeared in fruits grown in the field during the summer of 1927. At present the eight F_1 hybrid plants are vigorously growing in the greenhouse, but are too young to make their description of any value.

C. MAXIMA x *C. PEPO*

Parents	Number Pollinations	Fruits	Fertile Seeds
Hubbard x Connecticut Field	176	22	0
270 x 175	285	13	0
20 x 175	434	38	11
Marblehead x Connecticut Field	142	14	0
Victor x Connecticut Field	38	7	0
Delicious x Connecticut Field	57	5	0
20 unpollinated (flowers tied without pollinating)	43	0	0
	1175	99	11

These eleven 20 x 175 hybrid seeds appeared:

In the field, 1924, 4 seeds in 1 fruit
1925, 3 seeds in 2 fruits
1926, 2 seeds in 2 fruits

In the greenhouse, 1924, 2 seeds in 2 fruits.
The resulting F_1 hybrid plants were all grown in the greenhouse.

No. 20, a pure line of Hubbard squash and the pistillate parent of the hybrids, is characterized by rough heary stems, leaf blades and leaf stalks; rather kidney shaped leaves with rounded lobes and indistinct sinuses between the lobes. The fruit stalk is cylindrical and spongy. Shape of the fruit nearly cylindrical, but pointed at blossom end. Surface bumpy, color glossy dark green. Shell hard at maturity. Size 9 x 6 inches, weight about 6 pounds.

No. 175, a pure line of Connecticut Field Pumpkin—the staminate parent of the hybrids—has spiny stems, leaf blades and leaf stalks; the leaves are strongly lobed and have deep sinuses between the lobes. Fruit stalk hard at maturity, five-sided, distinctly grooved, not noticeably enlarged at attachment to fruit. Fruit round, flattened at both ends. Surface smooth, color orange yellow, shell thin and hard. Size about 16 x 10 inches, weight about 20 pounds.

From the eleven F_1 hybrid seeds of 20 x 175 only four plants grew to maturity, seven of the seeds giving rise to feeble plants which died when from four to six inches tall. These four plants were the progenies of two 20 x 175 fruits, each from a different plant; three of the seeds having come from one fruit and the fourth seed from another. The single hybrid plant

from the first fruit has been designated as No. 100 and the three hybrid plants from the second fruit as No. 101.

Three cuttings were made from No. 100 and the three resulting plants were grown in the greenhouse. No. 100 is characterized by prickly stems, leaf blades and leaf stalks. Leaf blades distinctly lobed with deep sinuses between the lobes. It will be noted that these vegetative characteristics are very similar to those of the staminate parent, No. 175. These plants bore very few flowers, either staminate or pistillate. All the pollen was abortive, and examination with the microscope showed it to be badly shriveled. Although self pollination of No. 100 resulted in no fruits, several fruits were secured by back crossing, as will be seen from the following table. These fruits were nearly spherical in shape, varied greatly in size, had hard, thick, warty shells and in color were dark green with a faint gray mottling. The seed coats and fruit stalk closely resembled those of *C. pepo* (175). None of these fruits contained any fertile seeds.

The above description of No. 100 will also serve for No. 101, with the exceptions that the terminal lobe of the No. 101 leaf was much shorter than that of No. 100, and the fruit of No. 101 was light green, decidedly mottled with gray and yellow, as opposed to the dark green and faint gray mottling of No. 100. The three No. 101 plants were very similar to each other in all respects.

POLLINATION OF F_1 HYBRIDS OF C. MAXIMA x C. PEPO

Parents	Number Pollinations	Fruits	Fertile Seeds
100 Selfed	6	0	0
100 Open pollinated in field		3	0
100 Unpollinated (flowers tied without pollinating)	12	0	0
100 x 175	6	2	0
100 x 20	3	1	0
20 x 100	5	0	0
101 Selfed	9	0	0

The small number of pollinations recorded for this first hybrid generation is due to the few flowers formed on these plants.

C. PEPO x C. MOSCHATA

Parents	Number Pollinations	Fruits	Fertile Seeds
175 x 5	134	14	37
Conn. Field x Striped Cushaw	24	1	Many
Early White Bush Scallop x Large Cheese	3	1	0

The pistillate parent in the 175 x 5 cross has been described earlier in the paper. No. 5, the staminate parent, has soft hairy stems and leaves. The leaf blades are weakly lobed without notches between the lobes, and have silvery spots at intersections of veins. Fruit is round, flattened at both ends, giving the appearance of a cheesebox. Shell thin, but hard,

smooth. Colory, creamy buff. Fruit stalk hard, five-sided, deeply grooved and distinctly flaring at attachment to fruit.

The hybrid seeds obtained from Conn. Field x Striped Cushaw were planted in the greenhouse and gave rise to vigorous plants, which are not yet sufficiently mature for satisfactory description.

The F_1 plants of 175 x 5 (designated as No. 125) were grown in both greenhouse and field. The shape of the leaf and the degree of prickliness of leaf and stem were intermediate between those of the parents. The fruit shape is short oblong, and the color a network of green over a light orange background. The network, incompletely covering the background, gives to the fruit the appearance of being splotched. These fruit characteristics are difficult to understand when we consider the shape and color of both parents. The shell of fruits is hard, smooth or slightly warted. Fruit stalk is flaring at attachment as in the staminate parent. Self pollinating No. 125 shows this F_1 generation to be very fertile, as many viable seeds were secured out of the 12 fruits resulting from 19 pollinations.

From these seeds a large number of F_2 plants were grown. Since it was not primarily the purpose to report the inheritance of characters in these crosses, no details will be given on the F_2 generation except that it was somewhat fertile, for out of 62 self pollinations of the F_2 (designated as No. 127) 19 fruits with many viable seeds were secured.

C. MOSCHATA x *C. PEPO*

Parents	Number Pollinations	Fruits	Fertile Seeds
Large Cheese x Conn. Field	294	18	0
Striped Cushaw x Connecticut Field	.62	18	0
5 x 175	31	1	0
	367	37	0

The failure to secure any fertile seeds of *C. moschata* x *C. pepo* is very surprising, in view of the large number of fertile F_1, also fertile F_2, seeds secured in the reciprocal pollinations.

C. MAXIMA x *C. MOSCHATA*

Parents	Number Pollinations	Fruits	Fertile Seeds
Hubbard x Large Cheese	3	1	0
270 x 5	2	1	Many
20 x 5	62	23	Very many
Pollination of F_1 hybrids			
15 Selfed	2	0	0
15 Unpollinated (tied up without pollinating)	63	0	0
15 x 20	87	18	Few
15 x 5	103	28	Few
20 x 15	13	2	0
5 x 15	5	0	0

Of all attempts to cross species, *C. maxima x C. moschata* proved to be the least difficult, it being possible to secure hybrids at will. The F_1 plants of 20 x 5, (designated No. 15), showed remarkable hybrid vigor. The stems and leaves were rough hairy, leaf blades intermediate in shape between those of No. 20 and No. 5, with silvery spots at intersections of veins. Pistillate flowers abundant, staminate flowers very few and sterile. Numerous fruits were secured, however, by back crossing and open pollination. The fruits were nearly spherical in shape. Shell thick, hard, bumpy, of uniform dark green color. Fruit stalk hard, five-sided, grooved, but not flaring at attachment.

No. 18, secured by pollinating 15 x 20, and No. 19, resulting from 15 x 5—both back crosses—were grown in the field. It is apart from the pupose, however, to give details concerning the characteristics of these back crosses.

C. MOSCHATA x *C. MAXIMA*

Parents	Number Pollinations	Fruits	Fertile Seeds
5 x 20	8	2	0
Large Cheese x Hubbard	192	41	Many
Striped Cushaw x Victor	1	1	0
Striped Cushaw x Marblehead	48	25	1
	249	69	Many

The small number of pollinations of the pure lines 5 x 20 is due to No. 5 flowering very late in the season—too late to make pollination worth while.

The single seed resulting from Striped Cushaw x Marblehead produced a plant which is now growing in the greenhouse but is not sufficiently mature to make determination of definite characteristics possible. The hybrid seeds resulting from Large Cheese x Hubbard have not yet been grown.

DISCUSSION

In planning the above investigations considerable thought was given the method to be used in protecting the flowers from contamination by foreign pollen. Several investigators in the field have isolated by tying a string or wrapping soft wire around the tips of the buds; others have made use of paper or parchment bags for this purpose. Both Lotsy (8, 9) and Vavilov (12) have criticized the former method, maintaining that it does not entirely exclude the possibility of the entrance of insects. The writer's experience has been that both methods are equally unsafe in careless or inexperienced hands, and that perfect isolation is secured by tying the corollas if proper care is exercised. The writer chose to protect against contamination by tying the corolla tips, rather than by the paper bag method, as the exclusion of light and interference with the circulation of air in the latter method are very possible limiting factors in crossing species, even though this is not the case in crossing varieties. In this connection it is worthy of note that both Lotsy and Vavilov are the only work-

ers who report having used the paper bag method, and neither secured hybrid seeds.

The phenomenon of parthenogenesis reported by the Hagedoorns (6), in which they secured fertile seeds from unpollinatd F_1 interspecific and intervariety hybrids, has been reinvestigated by Lotsy. He covered a considerable number of unpollinated female flowers on intervariety hybrid plants with paper sacks, but in not a single case did he obtain seeds.

Vavilov enclosed in parchment bags a number of female flowers of various genera and species of the Cucurbitaceae, but was unable to secure parthenogenetic seeds. It should be noted, however, that his work differed from that of the Hagedoorns in that he did not use hybrid plants. The writer made no systematic effort to study parthenogenesis in Cucurbita, but some data have been accumulated. With No. 20—a pure line of Hubbard squash—43 female flowers were tied without being pollinated. No fruits were obtained. In No. 100—the F_1 hybrid of 20 x 175—12 unpollinated female flowers were tied, but no fruits developed. Sixty-three unpollinated female flowers of No. 15—the F_1 hybrid of 20 x 5—were isolated in a similar manner, but no fruits resulted. While these data are quite meager, nevertheless so far as they go they fail to confirm the results of the Hagedoorns.

While a considerable number of hybrid seeds were secured in the above investigations, it is evident many of the pollinations produced nothing more than parthenocarpic fruits. This is in harmony with the results obtained by other workers in the field. It was observed, however, that when such parenocarpic fruits were stored they decayed much more quickly than did fruits containing fertile seeds stored under identical conditions. An examination of the interior of parthenocarpic fruits invariably revealed the presence of many well formed seed coats which were either collapsed or inflated, and without embryos; in some cases, however, small, partially developed and undifferentiated embryos were observed, although these were too rudimentary to produce plants.

CONCLUSIONS

1. The literature dealing with interspecific hybrids in the genus Cucurbita is reviewed and summarized.

2. A large number of cross pollinations between the three annual cultivated species of Cucurbita—*C. pepo, C. maxima* and *C. moschata*—with the results obtained therefrom, are reported. Both pure lines and commercial varieties were used in the investigation.

3. Using *C. pepo* as the pistillate and *C. maxima* as the staminate parent, eight hybrid plants were secured, the fertility of which has not yet been investigated. Reciprocally, the result was eleven fertile hybrid seeds, all the F_1 plants of which were entirely self sterile. Fruits developed on these plants only as the result of back crossing and open pollination, and in no

4. *C. pepo* x *C. moschata* gave rise to many seeds; both the F_1 and F_2 were quite fertile, a considerable number of viable F_3 seeds resulting. *C.* case did a fruit contain viable seeds.
Moschata x *C. peppo,* however, yielded no viable seeds.

5. No difficulty was experienced in crossing *C. maxima* with *C. moschata.* Without exception the F_1 was self sterile on account of abortive pollen. But it was found possible to procure fruit containing viable seed

from the F_1 plants by back crossing. Reciprocally, more effort was required, although the hybrids were fairly numerous. The fertility of the F_1 has not yet been studied.

6. Parthenocarpy was observed to be quite common, but in no instance was parthenogenesis observed.

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ARITHMETICAL CHANGES IN STATISTICAL CONSTANTS DUE TO CODING AND THEIR CORRECTION

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The process of coding as presented herein is a process by which, for expediting the calculation of statistical constants, more or less fictitious values are substituted for the observations without sacrificing their identity. It is the equivalent of the usual grouping into classes and translating the origin of measurements, which process, under the head of grouping, has been discussed by many writers on statistics including G. Udney Yule (1916), Truman L. Kelley (1923), Horace Secrist (1925), and R. A. Fisher (1925).

It seems to be rather universally agreed that grouping is a legitimate process, at least so far as the frequency distribution is concerned, but there is no agreement as to the exact process of grouping. The number of groups or class intervals and the class limits are doubtful points. Yule (1916) says that the number of classes should lie between 15 and 25 and that less than 10 classes leads to very appreciable inaccuracy; Kelley (1923) says that there should not be less than 12 classes; while Fisher (1925) says that the class interval should not be less than one-fourth the standard deviation, which is the equivalent of saying that there should be not less than 24 classes, since the total range of a variable is very approximately six times the standard deviation. Secrist (1925) says on page 168, "In writing the limits of groups, a smaller fraction of the whole unit should not be used than was employed in the actual process of measurement." Whereas Yule (1916) says on page 81, "The difficulty may always be avoided if it be borne in mind in fixing the limits to class intervals, these being carried to a further place of decimals, or a smaller fraction, than the values in the original record."

Before entering upon the problem illustrating the arithmetical changes in the various statistical constants, we will present the method of coding used. Only continuous series or discrete series in which the range is large in respect to the unit of measurement should be coded. Anyone may easily code a series by complying with the following five rules and one caution:

1. Determine the actual range of the series from the data and decide the number of class intervals into which the series is to be divided.

2. Divide the actual range by the number of classes and select a convenient class interval larger than the quotient.

3. Multiply this selected class interval by the number of classes and from this theoretical range subtract the actual range, take one-half of this difference, and subtract it from the least observation to secure the lower limit of the first class interval.

4. Add one-half of the selected class interval to the lower limit of the first class for the first class mark.

5. Set up the coding equation as follows:

$$\text{Observation (coded)} = \frac{\text{Observation} - \text{First class mark}}{\text{Class interval}}$$

Caution: Examine the coded series carefully to make sure that if there is a clustering of observations it will be about the mid-point or center of the class interval and not at the limits. If there is a clustering at the limits, the coding must be done over with a different class interval because the mid-point or class mark is substituted for the actual values, thus introducing inaccuracies which are inversely proportional to the density of grouping about the class mark.

The set of data used in this illustration consists of the total intelligence test score (I), an individual intelligence test score (G), total high school average (H), high school average in one subject (R), and first quarter college grade (Q), of 492 Iowa State College freshmen. Test G is a part of test I and average R is a part of average H. These were selected in this manner so as to insure some high correlations. The first quarter college grade (Q) is the dependent variable.

Each variable was coded into 10 classes and then into 20 classes. The coded values and the original observed values were punched on data cards for use in sorting and tabulating machines as shown in Plate I. With the aid of the sorting and tabulating machines and of calculating machines, the means, standard deviations, simple correlation coefficients, and multiple correlation coefficient were calculated following the tabular form presented by H. A. Wallace and George W. Snedecor (1925). The means and standard deviations of the coded sets are expressed in the code units and must be decoded, that is, expressed in the original units. In this illustration the means are adjusted by multiplying by the class interval and adding the first class mark. The standard deviations are adjusted by multiplying by the class interval. The decoded values of the constants and their probable errors are given in Table I.

Assuming that the values of the constants obtained from the uncoded data are correct, the values obtained from the coded sets differ from them as was expected. In this example, the differences are not great enough to cause any serious difference in interpretation, but the fact that they occur warrants investigation as to the possibility of correction. The correction to be used is, of course, the commonly used Sheppard's correction. Concerning the advisability of using Sheppard's correction, Fisher (1925) on page 150 says, "....., and the full effects on the distribution in random samples of using Sheppard's correction have never been fully examined, but there can be little doubt that Sheppard's correction should be used, and that its use gives generally an improved estimate of the correlation."

The tabular calculation of the correlation coefficients given by Wallace and Snedecor (1925) makes it possible to use Sheppard's correction with only a minor change. Five lines are provided for each variable instead of four. The first column appears as follows:

Sums	ΣA
Means	M_A
A_1	ΣA^2
A_2	$(\Sigma A)M_A$
A_3	$\Sigma A^2 - (\Sigma A)M_A$
A_4	$\Sigma A^2 - (\Sigma A)M_A - n/12$
A_5	$\sqrt{\Sigma A^2 - (\Sigma A)M_A - n/12}$

The leading entry in each block of lines is made to comply with the one above, the entry in line 4 being the usual entry in line 3 diminished by Sheppard's correction n/12. The subsequent entries in any block of lines is exactly as given by Wallace and Snedecor (1925) except that line 5 provides a place to enter the simple correlation coefficients. It is evident from the above illustration that Sheppard's correction does not affect the mean.

Those constants that are affected by Sheppard's correction are given in Table II in decoded form. The values in the column headed uncoded are the same as those in the same column in Table I, but are repeated here for comparison. From this table we find that Sheppard's correction has adjusted the value of each constant so that it lies within the probable error of the value secured from the uncoded set except the standard deviation of I (σ_1). This discrepancy is probably due to the wide range of I (from 57 to 277).

It appears from the above results that it is advisable to use Sheppard's correction if we desire to get the estimate which most closely approximates the value secured from the uncoded data. On the other hand, if we take the more usual statistical view that the present set of data is merely a random sample from a population and that the values secured for the various constants are approximations to the real values, the arithmetical changes due to coding are not large compared to the errors of random sampling so that the use of Sheppard's correction is not imperative. In fact, Sheppard's correction should seldom be used with small samples. As Fisher (1925) says on page 150, "The fact that with small samples the correlation obtained by the use of Sheppard's corection may exceed unity, illustrates the disturbance introduced into the random sampling distribution."

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TABLE I. The Statistical Constants and Their Probable Errors Without Correction.

Constant	Coded		Uncoded
	10 classes	20 classes	
M_I	142.2680 ± 1.0573	141.8265 ± 1.0397	141.5183 ± 0.8895
M_G	17.3488 ± 0.1337	17.2238 ± 0.1276	17.2093 ± 0.1299
M_H	86.9818 ± 0.1416	86.5651 ± 0.1427	86.4370 ± 0.1412
M_R	86.2276 ± 0.1652	86.2276 ± 0.1625	86.0935 ± .1646
M_Q	78.9938 ± 0.2873	78.8422 ± 0.2857	78.8496 ± 0.2813
σ_I	34.7688 ± .7475	34.1918 ± .7351	29.2512 ± .6289
σ_G	4.3963 ± .0945	4.1950 ± .0902	4.2732 ± .0919
σ_H	4.6896 ± .1008	4.6920 ± .1009	4.6444 ± .0999
σ_R	5.4323 ± .1168	5.3638 ± .1153	5.4133 ± .1164
σ_Q	9.4466 ± .2031	9.3940 ± .2020	9.2495 ± .1989
r_{IG}	.5989 ± .0195	.6256 ± .0175	.6267 ± .0185
r_{IH}	.4218 ± .0250	.4403 ± .0245	.4388 ± .0245
r_{IR}	.4279 ± .0249	.4433 ± .0242	.4397 ± .0245
r_{IQ}	.4602 ± .0240	.4666 ± .0240	.4689 ± .0237
r_{GH}	.2364 ± .0285	.2647 ± .0282	.2671 ± .0282
r_{GR}	.2210 ± .0292	.2266 ± .0288	.2266 ± .0288
r_{HQ}	.2629 ± .0282	.2745 ± .0279	.2795 ± .0280
r_{HR}	.8270 ± .0095	.8503 ± .0078	.8557 ± .0081
r_{HQ}	.4670 ± .0240	.4836 ± .0234	.4837 ± .0233
r_{RQ}	.4221 ± .0250	.4321 ± .0248	.4356 ± .0246
$R_{Q\cdot IGH}$	.5503 ± .0212	.5603 ± .0208	.5619 ± .0208

TABLE II. The Statistical Constants and Their Probable Errors After Sheppard's Correction

Constant	Coded		Uncoded
	10 classes	20 classes	
σ_I	34.0656 ± .7324	31.0716 ± .6680	29.2512 ± .6289
σ_G	4.3212 ± .0929	4.1769 ± .0898	4.2732 ± .0919
σ_H	4.6089 ± .0991	4.6745 ± .1005	4.6444 ± .0999
σ_R	5.3715 ± .1155	5.3486 ± .1150	5.4133 ± .1164
σ_Q	9.3078 ± .2001	9.3590 ± .2012	9.2495 ± .1989
r_{IG}	.6219 ± .0186	.6308 ± .0183	.6267 ± .0185
r_{IH}	.4380 ± .0246	.4440 ± .0244	.4388 ± .0245
r_{IR}	.4417 ± .0245	.4467 ± .0243	.4397 ± .0245
r_{IQ}	.4767 ± .0235	.4706 ± .0237	.4689 ± .0237
r_{GH}	.2447 ± .0286	.2669 ± .0282	.2671 ± .0282
r_{GR}	.2274 ± .0288	.2283 ± .0288	.2266 ± .0288
r_{GQ}	.2715 ± .0282	.2767 ± .0281	.2795 ± .0280
r_{HR}	.8510 ± .0084	.8559 ± .0081	.8557 ± .0081
r_{HQ}	.4843 ± .0233	.4872 ± .0232	.4837 ± .0233
r_{RQ}	.4332 ± .0247	.4349 ± .0247	.4356 ± .0246
$r_{Q\cdot IGHR}$	.5620 ± .0208	.5643 ± .0207	.5619 ± .0208

PLATE I.

Serial No.	I			G			H			R			Q		
	10	Uncoded	20	10	Uncoded	20	10	Uncoded	20	10	Uncoded	20	10	Uncoded	20
0 0 0	0	0 0 0	0 0	0	0 0	0 0	0	0 0	0 0	0	0 0	0 0	0	0 0	0 0
1 1 1 2 2 2 3 3 3	1 2 3	1 1 1 2 2 2 3 3 3	1 1 2 2 3 3	1 2 3	1 1 2 2 3 3	1 1 2 2 3 3	1 2 3	1 1 2 2 3 3	1 1 2 2 3 3	1 2 3	1 1 2 2 3 3	1 1 2 2 3 3	1 2 3	1 1 2 2 3 3	1 1 2 2 3 3
4 4 4 5 5 5 6 6 6	4 5 6	4 4 4 5 5 5 6 6 6	4 4 5 5 6 6	4 5 6	4 4 5 5 6 6	4 4 5 5 6 6	4 5 6	4 4 5 5 6 6	4 4 5 5 6 6	4 5 6	4 4 5 5 6 6	4 4 5 5 6 6	4 5 6	4 4 5 5 6 6	4 4 5 5 6 6
7 7 7 8 8 8 9 9 9	7 8 9	7 7 7 8 8 8 9 9 9	7 7 8 8 9 9	7 8 9	7 7 8 8 9 9	7 7 8 8 9 9	7 8 9	7 7 8 8 9 9	7 7 8 8 9 9	7 8 9	7 7 8 8 9 9	7 7 8 8 9 9	7 8 9	7 7 8 8 9 9	7 7 8 8 9 9

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