THE RESISTANCE OF DROSOPHILA TO ALCOHOL

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I

The interpretation of data relating frequencies or speed of activities and processes in living organisms to the temperature of the organism may be assisted by the examination of phenomena which are deliberately selected and experimentally made to be of a known kind of complexity. It has been supposed, not infrequently, that the activities of living cells and cell aggregates are inherently so complex that simple, reproducible, comprehensible relationships to magnitudes of a known, controlled relevant variable are not to be expected (*cf.* Clark, 1933). A direct way to test this notion is to examine phenomena which are known to be complex (*e.g.*, Crozier and Stier, 1925–26). If in terms of a type of analysis thought to give simple understandable results in "simple" cases, simplicity should apparently be revealed also in cases known to be complex, the method of interpretation might well be open to suspicion. On the other hand, it is perfectly obvious from the analysis of variation of performance in organic activities (Crozier, 1929; 1935) that the assumption of "complexity" in the sense indicated is not only gratuitous, but is inadmissible.

Examples of the complex type of situation with which one may attempt to deal are found in a number of cases where experiments have been made seeking to determine the temperature coefficients for the actions of drugs. It is clear that for fruitful treatment one must be able to dissociate (1) the relation between temperature and the time course of the action of the reagent employed, in terms of the organic performance which is measured, from (2) the effect of temperature upon the organic activity itself (Crozier and Stier (unpublished data); Crozier, 1934–35).

A further instance, which also appealed to us as possibly opening

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avenues for genetic experimentation, was suggested by the experiment reported in summary by Pearl, White, and Miner (1929). As a contribution to the estimation of the physiological nature of senescence they measured the times required for adult *Drosophila* of a particular strain to become "anesthetized" by the vapor of ethyl alcohol as a function of age and of sex, at 25.5°C. Their discussion of the results makes it clear that they considered the measured times to reflect the way in which the age of the organism expresses itself as determining the capacity to continue "living." A means might then be available for the physiological characterization of races in this respect. The endpoint for the observations is said to have been "a definite end stage of anaesthesia;" just what this was is not stated, and we have not been able to learn precisely what it was; our observations indicate that reversible anesthesia by ethyl alcohol does not provide really satisfactory endpoints for judgment of a given stage of the progress of narcosis. Between ages 1 and 60 days the times recorded for the achievement of anesthesia decline in an orderly fashion, from 9.6 to 4.1 minutes. They refer to these figures as measurements of "alcohol tolerance," and speak of the data as providing "an index or measure of physiological senescence—of the rate of growing old, in short—quite distinct and apart from the phenomenon of death." In this statement of viewpoint, and in the analysis offered for these data, the distribution of the occurrences of deaths in a population, with respect to age, is somehow thought of as being dependent upon an analogous distribution of the forces determining the length of life of a single individual, in the various individuals of a population, while these are still alive and for the most part quite remote from death. There is no reason whatever for supposing that the character of the age distribution of the incidence of deaths in a population can tell anything about, or be predicted from, the nature of the distribution of the magnitudes of the possibly relevant forces in the members of this population as obtained at an instantaneous cross-section of their lives. Certainly, there is no possibility of a formal statistical connection. The time course of change in these forces may have no direct relation to their intensities at a given moment, even supposing these intensities to be measurable; and we shall have occasion to demonstrate that mere survival time in alcohol is in no sense a direct measure of vital resistance; nor does it yield continuous curves as a function of age.

To the curve obtained by plotting time for anesthesia (y) against age in days (x), Pearl, White, and Miner fit the equation

$$y = 32.17 \, e^{-1.525 \, x^{1/4}}$$

for the combined data for males and females. The fit is certainly not "good." This formula they compare, however, with the Gompertz equation for the force of mortality:

$$\mu_x = Be^x,$$

saying that the difference in the form of the exponent is "unimportant from the standpoint of theory." The equation is also compared with du Noüy's expression for the time course of change of surface tension of blood serum—

$$y = y_o e^{-k \, x^{1/2}},$$

and they say that it gives "the same type of curve" because membrane phenomena may be involved in anesthesia. Aside from the specific forms of these comparisons, which are in point of fact mutually inconsistent, it is clear that the time for production of anesthesia in *Drosophila* by the vapor of alcohol is implicitly held to represent the time involved in a reaction between the living substance of the fly and the alcohol, at least in so far as the *shape* of the curve is concerned. The argument from the asserted similarity to the Gompertz curve is that the shape of the curve reveals the age course of the forces making for death, or conditioning "vitality."

If this type of interpretation is correct, it should be possible to investigate the way in which the reaction between *Drosophila* protoplasm and alcohol is affected by altering the temperature. This we have endeavored to test by a suitable series of experiments. It has been suggested, for example, that the temperature coefficients for protoplasmic activities are functions of age (Bĕlerádek, 1926; but *cf.* Crozier and Stier, 1926–27). It turns out that the shape of the curve relating time for effect of alcohol to age cannot possibly be related directly to the forces determining the incidence of mortality in a population. The analysis of a situation of this type requires that the nature of the constants implied in the descriptive curve be tested experimentally. This means that an additional coordinate of reference must be employed, permitting the assignment of physically

meaningful dimensions to the parameters involved. The variables concerned in the test are age of fly, sex, temperature, and vapor pressure of alcohol. It will be shown that from the data there can be obtained valid measures of the resistance of the fly to alcohol vapor, as a function of age,—but that they do not have the sort of significance suggested by Pearl. There is also secured a paradigm of procedure for the interpretation of certain types of curves connecting biological effect and temperature, and for the analysis of toxic effects.

II

At least three classes of variables are concerned in the killing of *Drosophila* by alcohol vapor, with reference to the way in which the time for death is modified when the temperature is altered. These must be separately examined and allowed for in a suitable way before one can decide whether the change in time-to-death as a function of age, with other conditions constant, reveals anything whatever as to the character of a presumed reaction between *Drosophila* protoplasm and the alcohol, or whether indeed the time-to-death is a legitimate measure of resistance to alcohol (or of tolerance). A *resistance* is to be measured only in terms of the relationship between the resistance and the force which it opposes; it does not have the dimensions of a time; to assume that it may be proportional thereto implies a mechanism of the effect observed, knowledge about which must be obtained by independent tests. The variables are: (1) the vapor pressure of the alcohol, (2) the speed of invasion of the body of the fly by the alcohol vapor, and (3) the reaction of the substance of the fly with the penetrating alcohol.

The flies used were of a Florida strain which had been inbred by pair matings through nineteen brother x sister generations. We are indebted to Dr. M. Demerec of the Carnegie Institution for a supply of the foundation stock. They were raised under uniform conditions of culture at 25°. The flies selected for the tests were also kept at this temperature until used. The experiments under consideration required series of sexually segregated flies ranging in age from 1 to 50 days. Each morning and evening at regular times all the flies were shaken out of the breeding bottles. They were then anesthetized (ether) and the sexes segregated, the males being placed in one fresh culture bottle, the females in another. Thus we had a group of male flies not more than 12 hours old, and a similar group of females. These were kept in an incubator for future use. At

the end of 50 days we had a series of segregated flies ranging in age from 1 to 50 days. Every 7 days the aging flies were shaken into new bottles containing freshly made food. In any cases of mold or bacterial contamination or of fertilized females the bottles as well as the flies were discarded.

When placed in air containing alcohol vapor a fly becomes after a variable latent period "intoxicated;" it staggers or flits about jerkily. This is followed by a stage of partial narcosis during which the fly lies on its back and is more or less immobile. Many flies continue to move their legs sporadically. Sometimes, after a fly has been on its back for several minutes it will right itself and stagger about again before collapsing. Occurrences of this type introduce a large uncertainty into the use of a condition of anesthesia as an end-point for judging the time of action of the vapor. The death-point is much more definitely recognizable. The prone
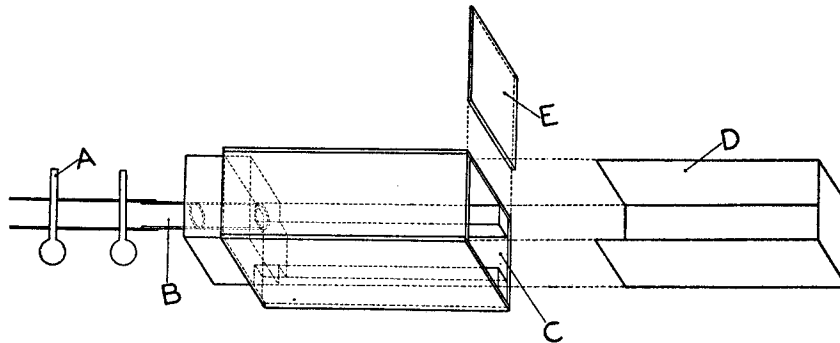

FIG. 1. Chamber in which *Drosophila* were exposed to alcohol vapor. *C*, trough containing absorbent paper saturated with alcohol; *D*, basket of wire screening, inserted on the shoulders at the sides of *C*; *E*, glass plate closing end of chamber; *A*, pinch-cocks on rubber tube, forming small air-lock for admission of flies; *B*, glass tube in cork plug.

fly suddenly flexes its previously extended legs, so that they are then interlocked. Before arriving at this stage, flies removed from the alcohol vapor will usually recover. After this movement has been exhibited, however, recovery does not occur. The drawing-in of the legs is precise and sudden, and provides a definite end-point.

The time elapsing between the introduction of a fly into alcohol vapor and its death was measured with a stop-watch, to the nearest 5 or 10 seconds. The usual procedure was to introduce a group of flies into a plate glass chamber in which the atmosphere was saturated with alcohol. The chamber (Fig.1) measured (inside dimensions) 13 × 22 × 60 mm. It contained on the bottom a trough for the accommodation of strips of paper towelling which had been soaked in redistilled "absolute" ethyl alcohol. Above the trough there was inserted a basket of brass wire screening. This effectively prevented the flies from getting

into contact with the wet towelling. The open end of the chamber was closed by a sliding glass plate, sealed with vaseline. The opposite end of the chamber carried a sealed-in cork plug. A glass tube extended through the cork into the chamber. Flies in groups of from five to fifteen were shaken through a funnel and rubber tubing into the glass tube. Two pinch-cocks on the rubber tubing produced a small intermediate chamber or air-lock. The flies were shaken from this air-lock into the alcohol chamber; after some experience this could be accomplished very quickly and neatly.
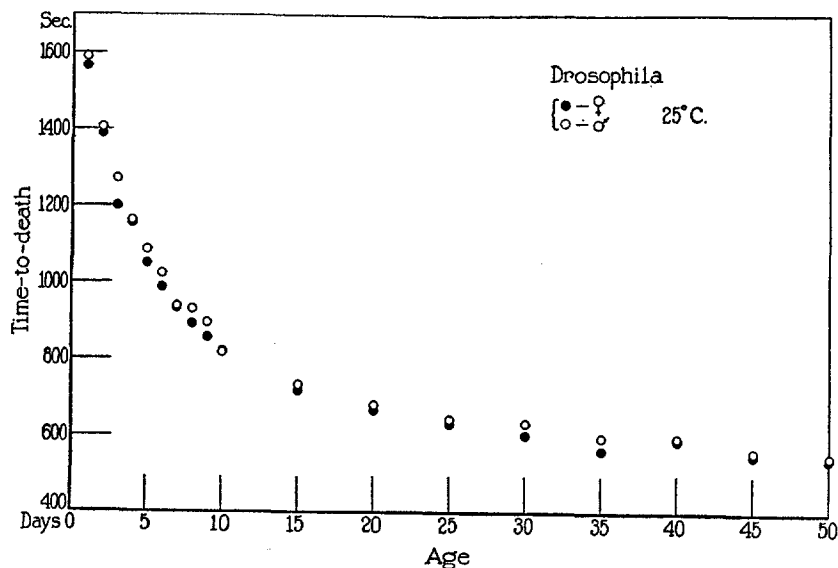

Fig. 2. Mean time-to-death for ♂ and for ♀ *Drosophila* in air saturated with vapor of ethyl alcohol at 25°C. (Table I). See text.

Usually the chamber was placed on the stage of a dissecting microscope for determination of the times-to-death. The procedure was repeated until observations had been obtained on thirty or more flies at each age (see tables), at each of the following temperatures: 10°, 15°, 20°, 25°, 30°, 35°C.

For determinations at temperatures below 25° the complete apparatus was moved into one or another of several constant temperature rooms, the temperature of which had been regulated to the desired level. The temperature was continually observed with a thermometer with the bulb as close as possible to the alcohol chamber. For determinations at 25°, 30°, and 35° the apparatus was placed in a large thermostatically controlled air bath with a plate glass top. Manipulation was possible through two apertures for the hands. The ocular

tubes of the microscope projected into the chamber through a hole in its top.   The temperature fluctuations did not exceed ±1° at the extreme.

Table I contains the results of determinations of time-to-death at 25°C. as a function of age, for males and for females, in an atmosphere saturated with alcohol vapor.   The course of the data (Fig. 2) is in a general way quite similar to that given by Pearl, White, and Miner (1929) for "anesthesia."   The time-to-death apparently declines in a

TABLE I

Time-to-death for *Drosophila* in air saturated with vapor of ethyl alcohol, at 25°C.   Each mean time is the average of about 30 measurements.

Age	♀♀		Time	♂♂	
days	*sec.* ±	*σ*		*sec.* ±	*σ*
1	1,566 ±	53.6		1,590 ±	79.6
2	1,389	79.9		1,407	58.3
3	1,200	73.0		1,272	71.0
4	1,158	69.6		1,165	80.0
5	1,052	79.2		1,088	60.5
6	989.0	67.4		1,025	53.4
7	934.0	82.7		938.3	71.4
8	893.8	68.2		932.3	68.8
9	857.7	63.5		896.0	80.0
10	822.1	60.6		818.3	63.8
15	718.4	62.2		734.6	76.1
20	666.8	62.4		681.7	60.8
25	633.0	69.8		645.0	73.6
30	604.7	70.2		635.8	74.2
35	564.6	75.9		597.3	86.7
40	590.0	86.6		596.3	78.4
45	552.1	77.5		561.2	76.8
50	542.8	83.5		549.2	78.2

rather orderly fashion with age, and is consistently longer for males than for females.   We do not find that the curve can be satisfactorily fitted by an equation of the type used by Pearl.   No smooth curve is drawn through these observations because, as will be shown presently, the succession of points is properly to be fitted by a line with sharp changes of curvature and is a composite affair.

To obtain from such observations a number which may be taken to

represent the resistance of the fly to alcohol requires the introduction of additional variables, in this case vapor pressure and temperature.

III

To cause death it may be presumed that a certain amount of alcohol must have entered the fly. This quantity, which we may call X, might be expected to appear as a function of age of fly, sex, temperature, and vapor pressure of alcohol. When the outer concentration of alcohol is unchanged by the diffusion of alcohol into the flies, a condition assured in the present case by the arrangements under which observation is made, the rate of diffusion at death is constant at each value of P and of X. The time t for attainment of X will then be a declining rectilinear function of P, for each value of X,—that is, for each age and sex,—on the assumption that X itself depends only on sex and on age, and is independent of P. X could appear to depend on P if a "defensive" reaction of the fly (involving its respiratory movements, for example) varied in over-all efficiency with P or t or both, but there is no indication of necessity for such a supposition. On this basis, the proportionality constant K is a coefficient of invasibility.

The essential rôle of physical diffusion of alcohol in this matter, to the practical exclusion of any effect for which the temperature might be held responsible, was tested in several ways. It was desired to compare the times-to-death at different temperatures for flies of the same age in atmospheres containing different partial pressures of alcohol. In a semi-quantitative way this was done by the following method.

A gas-collecting bottle was closed with a two-hole rubber stopper; through one hole extended a glass tube by which flies could be introduced, one at a time; through the other hole extended a tube which had been drawn out to a very fine tip. The pipette tube was partially filled with absolute alcohol; a rubber bulb closing the pipette made it possible to form at the tip successive droplets of alcohol of equal size. A known number of drops could then be released into the bottle; this was uniformly done at 24°C. During the formation of a droplet, of course, considerable evaporation of alcohol occurs, and the amount evaporated from a given droplet forming in the closed space will be increased by an amount which declines in a nearly exponential fashion with the number of droplets formed precedingly. Consequently, the vapor pressure of alcohol cannot be taken from the number of droplets released in the closed bottle; but in successive trials, atmospheres containing graded amounts of alcohol can be duplicated.

Table II contains the results of measurements made in this manner. It is perfectly clear that although the resistance of the flies does depend upon their age, and upon the vapor pressure of the alcohol, it is not distinguishably dependent upon the temperature; the small effect of a temperature coefficient of diffusion is masked by the inevitable

TABLE II

Time-to-death for *Drosophila* ($\sigma \sigma$ and $\circ \circ$, mixed) of two ages, with the P.E.'s ($n = 10$), in atmospheres containing different concentrations of alcohol, at each of two temperatures. See text; the concentrations are given arbitrarily in numbers of drops of alcohol introduced into a fixed volume of air under controlled conditions; the "number of drops" is not directly proportional to the vapor pressure of alcohol.

Age 1 to 6 hours		
Drops alcohol	$T = 15°$	$T = 30°$
	min.	*min.*
10	90.0 ± 1.07	93.9 ± 1.44
15	52.8 ± 1.58	54.6 ± 1.23
20	35.6 ± 0.677	37.2 ± 0.733
30		28.9 ± 0.592
40		21.9 ± 0.592
50 (saturation)		20.8 ± 0.621

Age 10 days		
Drops alcohol	$T = 15°$	$T = 30°$
10	57.2 ± 0.902	54.6 ± 1.13
15	35.2 ± 0.733	38.8 ± 0.818
20	24.1 ± 0.648	22.3 ± 0.762
30		13.6 ± 0.779
40		15.0 ± 0.733
50		12.4 ± 0.451

variations in resistance from one individual to another. The data of Table II are plotted in Fig. 3.

To test the correctness of this view, experiments were performed in which the time-to-death was obtained in the case of flies exposed at different temperatures to the vapor of ethyl alcohol saturated at 10°C. A vessel at 10°, containing an atmosphere saturated with alcohol vapor at 10°, was connected by a capillary tube with a chamber held at 10°, 20°, 25°, or 35°. It was assumed that, after diffusion had proceeded

for some time, the chamber at the higher temperature would contain alcohol vapor in equilibrium with liquid alcohol at 10°. The two chambers were of the same size. The data from this experiment are summarized in Table III. They show that in the case of flies 1 to 6 hours after hatching there is a slight but perhaps significant fall in the
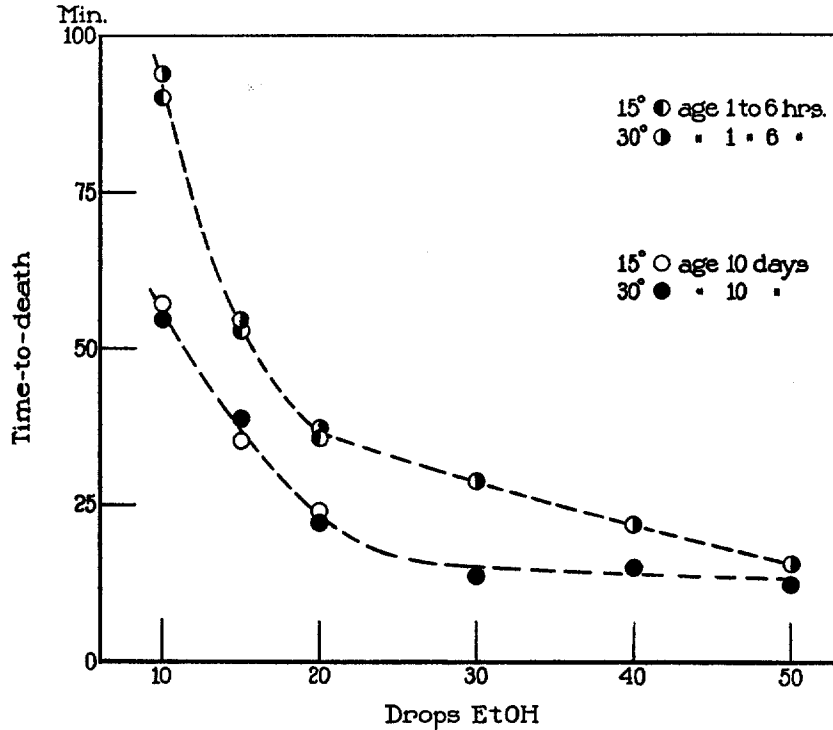

FIG. 3. Vapor pressure of alcohol (arbitrary scale of unequal units based upon "number of droplets of alcohol;" see text), rather than temperature, is the significant variable determining time-to-death in flies of a given age (Table II); both sexes.

time-to-death from 10° to 25°, with a slight, possibly significant, rise again at 35°; variation in the proportion of males to females in the sample could be responsible for the differences, or variations in age. With flies 15 days old, there is no difference in time-to-death at 10° and at 35°, with vapor pressure of alcohol the same. This is consistent with the conclusion already drawn that the change in time-to-death

is in by far the greater part determined by the vapor pressure of the alcohol in the atmosphere.   To only a very slight extent, if detectably, does it seem to be determined by the effect of temperature upon the invasion of the fly's body by the alcohol, or by any reaction of the fly connected with or influencing its respiratory movements.

We assume, then, from the foregoing tests, that the rate of invasion of the body of *Drosophila* by alcohol is the variable which determines the time of death in these experiments.   When dealing with saturated atmospheres, and obtaining different partial pressures of alcohol by varying the temperature, we may as a first approach consider only the differences in vapor pressure of alcohol.   We expect that in general, with flies of one sex and age, the time-to-death will be a rectilinear function of the vapor pressure of alcohol, although at the highest

TABLE III

Drosophila (σ σ and ♀ ♀) exposed at different temperatures to air saturated with alcohol vapor at 10°C.   Mean times-to-death, in minutes, with P.E.$_m$; n = 10.

Age of flies	Temperature, °C.			
	10	20	25	35
1 to 6 hrs.	37.3 ± 0.648	35.4 ± 0.621	33.5 ± 0.648	36.8 ± 0.564
15 days	17.0 ± 0.677			18.1 ± 0.536

temperatures (and partial pressures) some deviations from this would not be surprising.   The vapor pressure of ethyl alcohol has a rather high temperature coefficient; the vapor pressure (P) in equilibrium with liquid alcohol at absolute temperature T is described by the Clausius—Clapyeron equation

$$dP/dT = -(L/RT^2),$$

where L, the latent heat of vaporization, is 11,300 cals. per mol (Fig. 4). For any one age of fly, on the diffusion hypothesis, we may then translate the temperatures used into their equivalents in vapor pressure of alcohol, and expect to find that time-to-death (t) is a declining rectilinear function of P.   This is illustrated in Fig. 5 (data in Table IV), and the expectation is very satisfactorily met.   It is clear that the

criterion of a death-point used is one which can be employed in a consistent, unequivocal manner.

It is important to notice that the slope of the line in Fig. 5 is a valid index of the resistance of the flies ($\sigma\ \sigma$, 8 days) to alcohol vapor. The slope is conveniently expressed as $-\Delta t/(\Delta P=40$ mm.$)$, and in Fig. 5 it has the value -332 sec./40 mm. It is a real (though inverse) measure of *resistance* to alcohol, since, independent of P, and
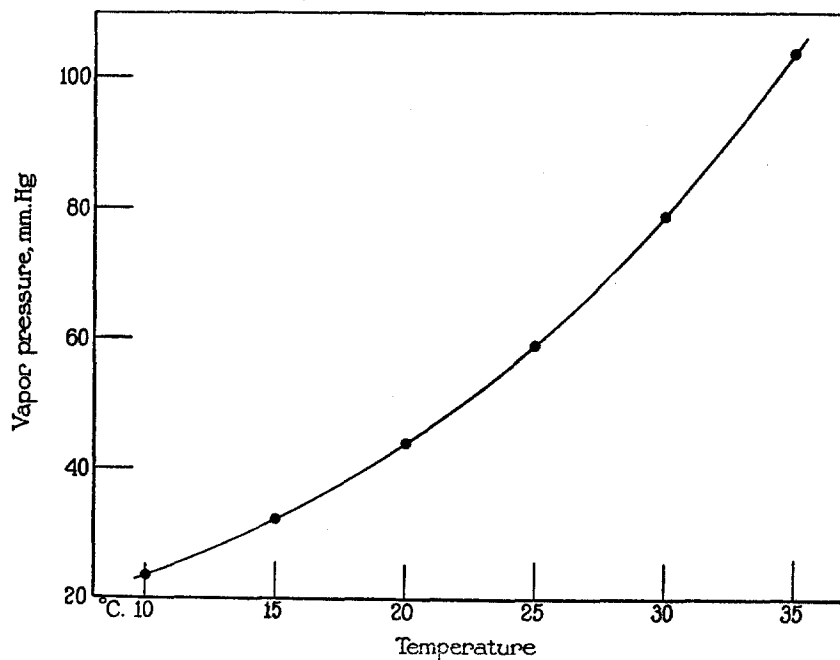

Fig. 4. The vapor pressure of ethyl alcohol as a function of temperature (data from International Critical Tables); the curve is that for $dP/dT = 11,300\ RT^2$.

of t, it measures the decline of time of survival per unit increase in concentration of alcohol vapor. This unit of *invasibility* we will call S. It is not to be confused with protoplasmic *susceptibility*; it says nothing about X, the minimum lethal dose of alcohol. The mere measure of time of survival does not give information of the same kind as S, and indeed its indications (as we shall show later) may be in this respect quite misleading.

IV

The resistance to penetration of alcohol depends in a systematic way upon age and sex. The observations of time-to-death are collected in Table IV. The data for females are plotted in Fig. 6, for males in Fig. 7. For reasons already considered the temperature is disre-
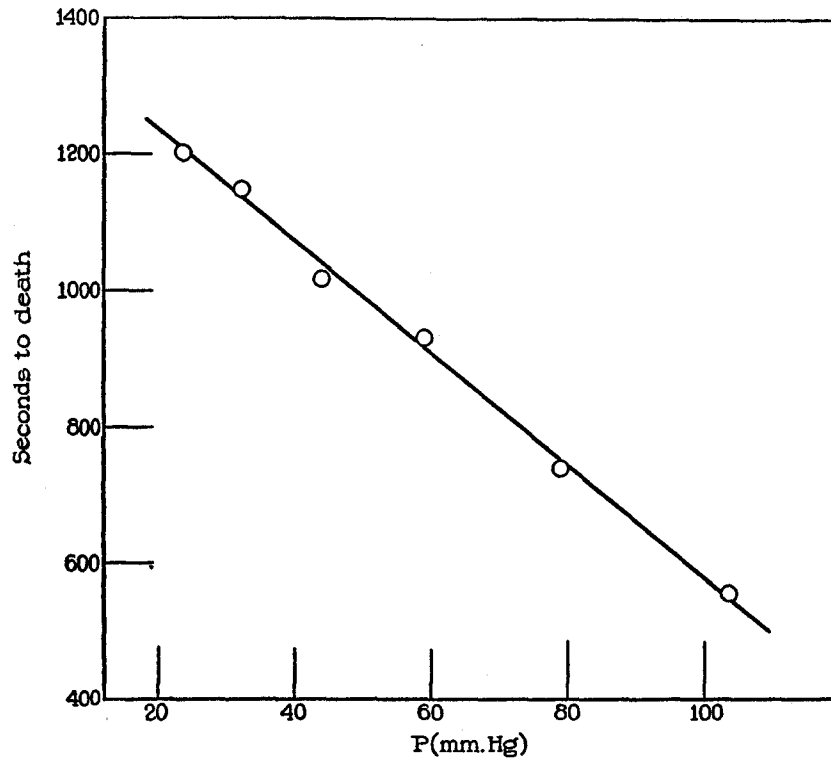

FIG. 5. The time-to-death is a declining rectilinear function of vapor pressure of alcohol, independent of the temperature used to maintain the vapor pressure at a given level (see text; Table IV); data for male flies, 8 days old.

garded; its only significance in the experiments is to regulate the vapor pressure of alcohol. At each age the time-to-death is a rectilinear function of the vapor pressure of alcohol. At the highest vapor pressure the flies 20 days old or older show a systematic decline in resistance to invasion of alcohol; this must be due primarily to the

TABLE IV

Time to death, sec., and σ_t

Mean time-to-death for males and females, at various ages (age in days ± 6 hours), with the standard deviations (σ_t) of these means, at 6 vapor pressures of alcohol.

Age	$P = 23.6$ mm. $T = 10°$	32.2 mm. 15°	43.9 mm. 20°	59.0 mm. 25°	78.8 mm. 30°	103.7 mm. 35°
days						
1 f	2,092.7 ± 101.4	1,934.4 ± 71.7	1,761.7 ± 75.7	1,565.8 ± 53.6	1,257.0 ± 75.1	921.0 ± 100.1
m	2,135.5 ± 97.1	1,980.7 ± 66.8	1,825.3 ± 80.2	1,589.7 ± 79.6	1,288.5 ± 66.6	956.1 ± 96.5
2 f	1,896.2 ± 86.7	1,768.2 ± 49.8	1,579.2 ± 77.2	1,389.4 ± 79.9	1,115.7 ± 74.2	803.0 ± 88.8
m	1,892.3 ± 85.1	1,771.9 ± 83.2	1,586.8 ± 89.8	1,406.9 ± 58.3	1,157.6 ± 76.1	840.0 ± 83.8
3 f	1,721.7 ± 105.5	1,576.2 ± 79.6	1,425.5 ± 94.0	1,200.0 ± 73.0	999.6 ± 74.3	768.9 ± 76.9
m	1,770.8 ± 95.6	1,575.0 ± 66.4	1,455.9 ± 78.0	1,272.1 ± 71.0	990.7 ± 79.0	746.6 ± 83.0
4 f	1,535.6 ± 75.4	1,470.0 ± 82.9	1,334.3 ± 73.4	1,158.3 ± 69.6	934.1 ± 73.0	701.0 ± 95.5
m	1,555.5 ± 84.0	1,448.7 ± 46.4	1,363.2 ± 69.7	1,164.6 ± 80.0	957.9 ± 71.0	665.7 ± 80.6
5 f	1,400.0 ± 77.1	1,339.1 ± 65.7	1,206.5 ± 80.7	1,051.9 ± 79.2	890.4 ± 82.5	630.0 ± 89.2
m	1,403.3 ± 58.7	1,356.9 ± 73.6	1,258.5 ± 81.4	1,087.6 ± 60.5	882.1 ± 72.8	631.6 ± 75.4
6 f	1,264.8 ± 56.4	1,211.6 ± 61.9	1,104.7 ± 80.8	989.0 ± 67.4	805.2 ± 78.8	589.3 ± 80.3
m	1,287.1 ± 78.0	1,207.7 ± 68.8	1,144.1 ± 66.7	1,025.4 ± 53.4	816.6 ± 70.2	620.7 ± 79.7
7 f	1,232.5 ± 73.2	1,144.0 ± 64.9	1,051.4 ± 86.2	934.0 ± 82.7	777.4 ± 68.4	593.3 ± 85.2
m	1,242.6 ± 74.5	1,149.1 ± 71.2	1,078.6 ± 58.5	938.3 ± 71.4	777.9 ± 66.4	564.5 ± 76.1
8 f	1,159.4 ± 71.8	1,100.3 ± 72.0	994.5 ± 72.4	893.8 ± 68.2	724.7 ± 66.3	516.7 ± 81.5
m	1,203.5 ± 88.8	1,149.4 ± 71.0	1,017.9 ± 84.4	932.3 ± 68.8	741.0 ± 89.1	559.0 ± 87.2

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9 f	1,100.0 ± 78.0	1,067.3 ± 92.3	971.7 ± 71.1	857.7 ± 63.5	715.5 ± 68.4	528.6 ± 76.1
m	1,096.7 ± 73.7	1,086.3 ± 102.1	1,013.8 ± 71.4	896.0 ± 80.0	721.8 ± 74.2	488.7 ± 82.0
10 f	1,059.7 ± 47.5	1,013.8 ± 72.3	930.0 ± 73.7	822.1 ± 60.6	603.9 ± 76.4	470.7 ± 78.8
m	1,110.6 ± 56.0	1,041.0 ± 80.7	947.0 ± 56.6	818.3 ± 63.8	635.2 ± 85.4	456.0 ± 88.6
15 f	958.2 ± 81.5	935.7 ± 85.7	802.8 ± 79.8	718.4 ± 62.2	591.3 ± 84.7	427.4 ± 85.1
m	983.6 ± 74.0	930.0 ± 92.4	811.3 ± 73.1	734.6 ± 76.1	631.4 ± 86.2	436.1 ± 72.5
20 f	850.0 ± 65.5	791.5 ± 63.9	739.7 ± 81.2	666.8 ± 62.4	571.6 ± 90.0	393.4 ± 45.5
m	855.8 ± 63.4	836.5 ± 84.8	768.6 ± 65.6	681.7 ± 60.8	618.2 ± 82.4	397.1 ± 50.9
25 f	802.7 ± 83.9	747.1 ± 65.4	699.1 ± 79.0	633.0 ± 69.8	542.4 ± 73.8	367.6 ± 36.6
m	821.7 ± 76.2	774.5 ± 75.3	705.0 ± 86.6	645.0 ± 73.6	581.1 ± 77.6	368.6 ± 30.5
30 f	791.2 ± 87.2	711.3 ± 77.1	660.3 ± 58.2	604.7 ± 70.2	499.7 ± 76.1	362.7 ± 28.3
m	757.7 ± 76.9	747.2 ± 73.7	668.7 ± 66.4	635.8 ± 74.2	553.9 ± 72.2	366.2 ± 38.7
35 f	809.2 ± 78.6	679.1 ± 81.5	639.0 ± 59.6	564.6 ± 75.9	491.0 ± 75.8	352.4 ± 43.4
m	713.6 ± 76.8	693.2 ± 86.4	667.1 ± 66.6	597.3 ± 86.7	512.7 ± 72.9	344.2 ± 44.1
40 f	678.7 ± 66.7	666.2 ± 74.7	599.7 ± 81.0	590.0 ± 86.6	487.6 ± 72.4	341.9 ± 46.2
m	713.2 ± 78.8	668.3 ± 89.1	620.0 ± 75.2	596.3 ± 78.4	506.2 ± 75.6	338.6 ± 93.5
45 f	679.1 ± 86.9	636.9 ± 89.8	609.1 ± 68.7	552.1 ± 77.5	472.7 ± 69.1	316.2 ± 39.6
m	686.3 ± 81.4	666.3 ± 91.8	634.1 ± 80.6	561.2 ± 76.8	483.2 ± 81.6	317.5 ± 29.8
50 f	653.9 ± 72.2	626.7 ± 69.4	611.9 ± 56.6	542.8 ± 83.5	469.6 ± 69.6	312.8 ± 47.3
m	676.6 ± 84.4	658.7 ± 66.4	602.8 ± 73.7	549.2 ± 78.2	480.0 ± 72.0	306.5 ± 40.6
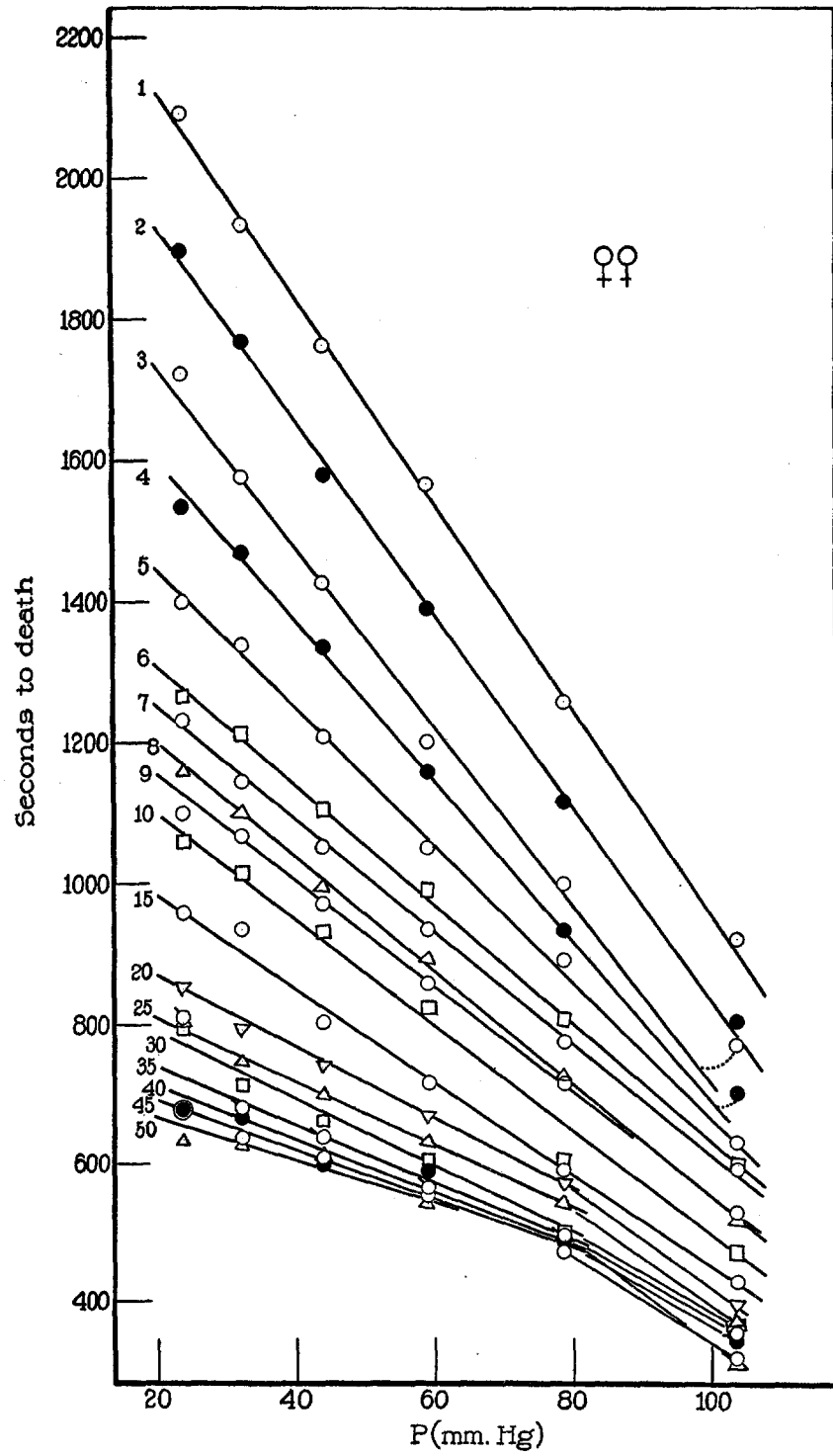
FIG. 6. Time-to-death as a function of vapor pressure of alcohol for female *Drosophila* at various ages after emergence; age in days indicated on each line.
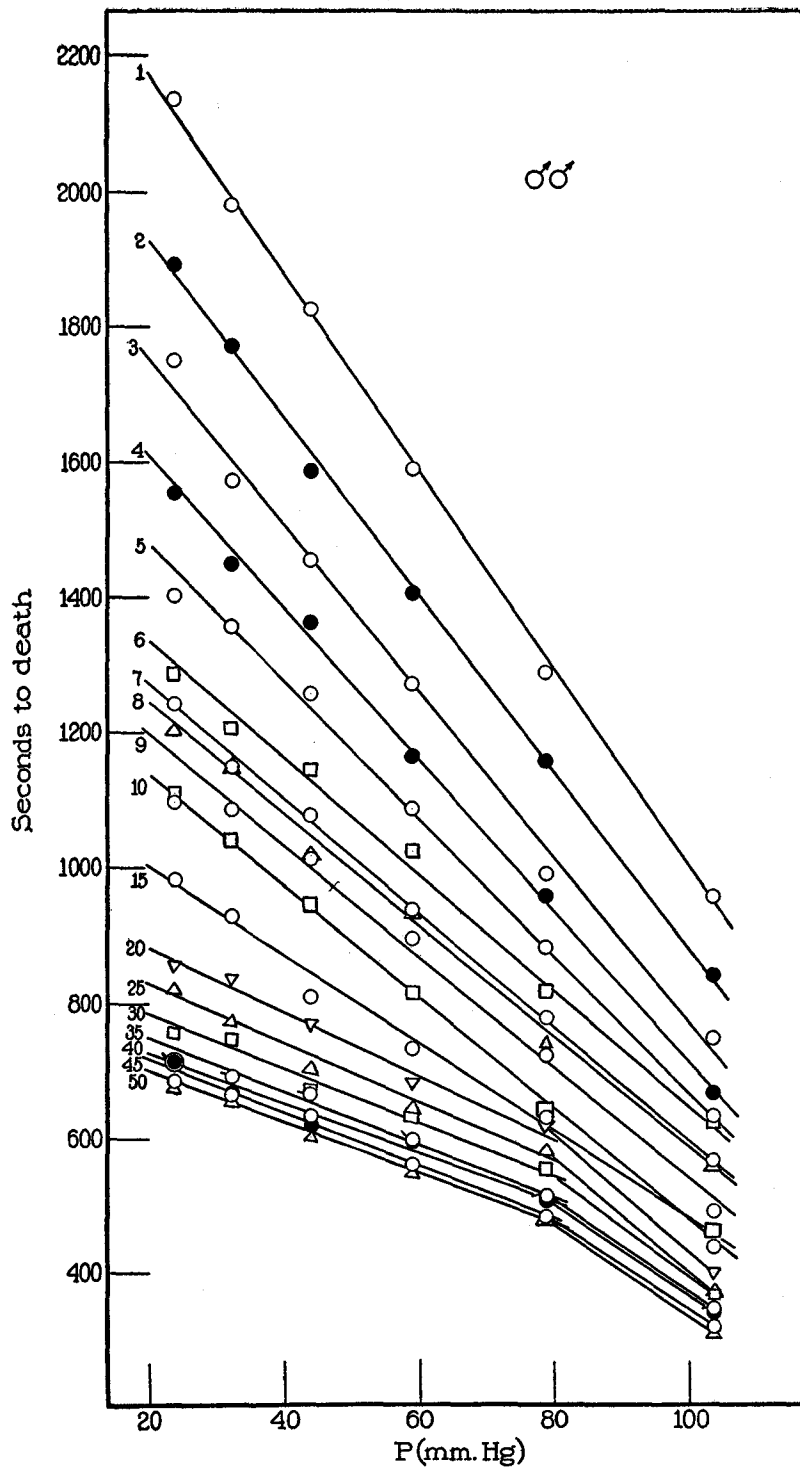
538

FIG. 7. Time-to-death as a function of vapor pressure of alcohol, males, at different ages.

539

influence of the high vapor pressure of alcohol, rather than to the temperature (35°), since at 35° flies in alcohol vapor saturated at 10° die after the "10°" time, showing no certain effect of the high temperature upon the killing process (cf. Table III).

The slope of the t, P graph decreases with advancing age. Since the ordinate position of the graph is higher the younger the animal, it is obvious that two criteria which might easily be employed for the estimation of resistance (or "tolerance") as a function of age give results which are in sharp contrast. The invasion of the fly by alcohol is easier the younger the imago, but it is clear that the quantity of alcohol required to kill is greater the younger the fly. The low temperature coefficient and the character of the t, P graph alike and independently show that the relation of t and P is determined by diffusion of alcohol into the fly; it cannot be supposed that the times observed at one value of P are capable of measuring the comparative "vital resistance" as a function of age, since the lethal quantity of alcohol (X) must be supposed to be determined only by age and by sex of fly, and not by P or by the temperature,—otherwise, the graphs in Figs. 6 and 7 could not be rectilinear.

The relationship between age and lethal dosage here observed is somewhat unusual. In silkworms, for example, given arsenic by mouth, the increment in $1/t$ per unit increase of dose is greater the younger the instar, while the minimum lethal dose *increases* with advancing age (Campbell, 1925-26).

It might be suggested that if one were to employ the view exploited by Pearl, White, and Miner (1929) a mortality curve could perhaps be obtained by extrapolation on the ordinate axis at $P = 0$. But this would be quite incorrect, for several reasons—chiefly because the observed times-to-death are "times-to-death in the presence of enough alcohol to kill," and extrapolation to lower partial pressures of alcohol than the minimum lethal could not give figures with dimensional significance.

Nor can it be supposed that the manner of change of resistance to alcohol as age advances could be obtained in another way, namely by finding from Figs. 6 and 7 the vapor pressure of alcohol required to kill at a constant time. Disregarding complications due to the fact that at constant P the shape of the curve for "killing" would probably depend

on the criterion of death employed (*cf.* Rahn and Barnes, 1932–33; Oster and Arnold, 1934–35), it is also true that the shape of the curve for P_t *vs. age*, where P_t is the vapor pressure of alcohol required to kill in t seconds, depends upon the time chosen, and is not smooth. These curves might have a simple significance if the various P, t graphs (Figs. 6, 7) did in fact converge upon one focus on the t axis; but they do not.

TABLE V

The slope of the curve relating time-to-death to vapor pressure of alcohol, as a function of age; the slope is $S = -\Delta t/(\Delta P = 40 \text{ mm.})$.

Age	S	
	f	m
days		
1	586	586
2	546	521
3	502	492
4	456	446
5	388	405
6	341	344
7	321	340
8	320	332
9	300	331
10	300	328
15	202	261
20	193	191
25	192	175
30	180	160
35	144	147
40	143	146
45	138	157
50	124	149

The slope constant S, computed from $S = -\Delta t/ (\Delta P = 40 \text{ mm.})$, is shown as a function of age in Table V and in Fig. 8. It is apparent that a continuous smooth curve cannot be used to describe these coefficients of invasibility. The reciprocals of S, $1000/S = R$, have the dimensions of change in partial pressure of alcohol required to produce a fixed alteration in time-to-death, and thus in the time required for the diffusion of a quantity of alcohol which is fatal (Fig. 9).
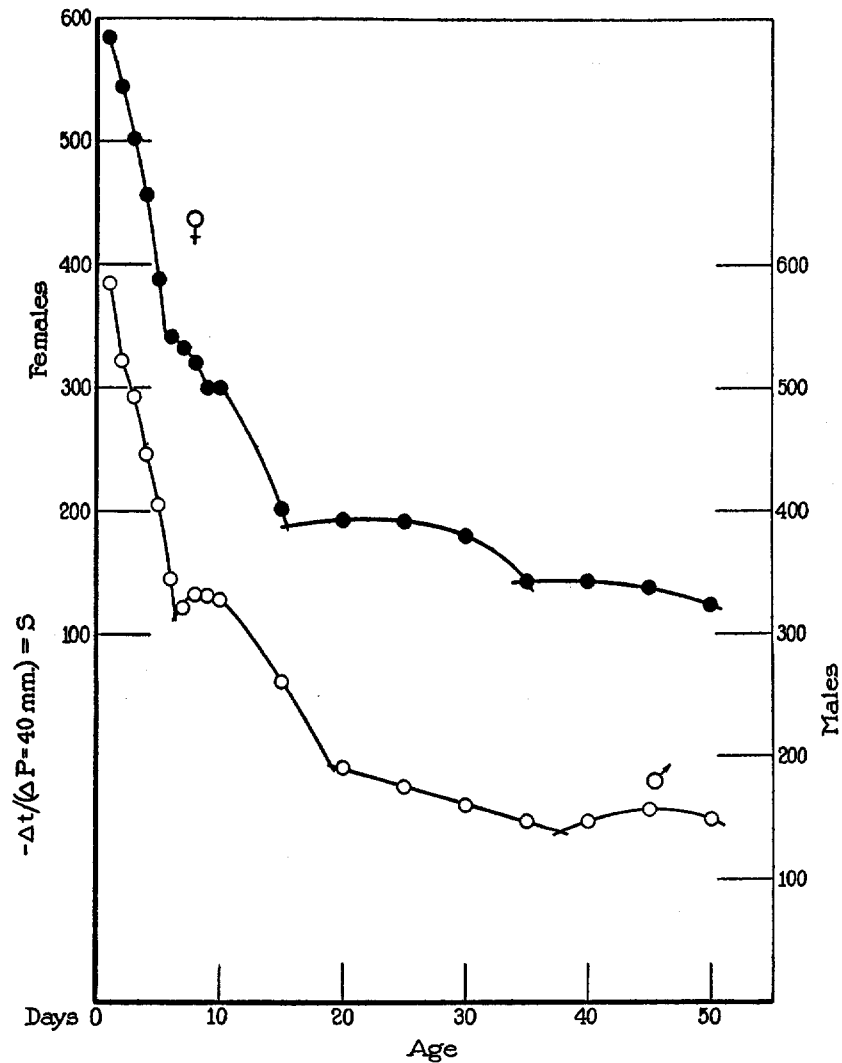
FIG. 8. The slopes of the lines in Fig. 6, $S = -\Delta t/(\Delta P = 40$ mm.$)$, which are measures of invasibility by alcohol, decline in a particular fashion for males and for females.

This latter curve also shows, of course, that the resistance of the fly to penetration by alcohol from its vapor increases as a function of age and exhibits successive cyclic variations. These fluctuations in relative

invasibility differ significantly in the two sexes; they are not entirely synchronous, although the general character and extent of the changes are similar in males and in females.

We can obtain independent evidence regarding cyclic fluctuations in resistance by extrapolating in the opposite direction. By extension of the lines in Figs. 6 and 7 to $t = 0$ we obtain estimates of the ideal vapor pressure required to kill instantaneously at each age. In this case the factor of invasibility is eliminated. In Table VI these values of P_0 are given, and they are plotted in Fig. 10. P_0 at ages above 20
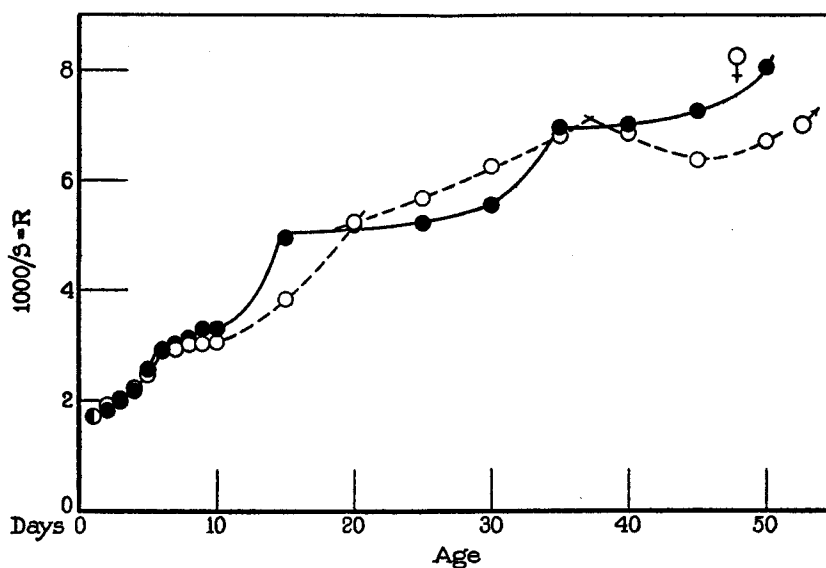

FIG. 9. The reciprocals of S (Fig. 8), measuring the resistance of *Drosophila* to invasion by vapor of ethyl alcohol, as a function of age. $R = 1000/S$.

days is estimated from the final slopes at the two highest values of P. It is apparent that the resistance is not a simple function of age, but goes through several maxima. It is also apparent that neither from the standpoint of invasibility (Figs. 8, 9) nor of resistance to the toxic influence of the alcohol (Fig. 10) can it be said that the males or the females are systematically the more resistant, despite the in general distinctly longer survival times of the males at any given vapor pressure of alcohol (Table IV).

If X, the internal concentration or amount of alcohol which is just fatal, changes with age and depends on the sex, then the time taken to produce X at constant P should be inversely proportional to S, the coefficient of invasibility, and proportional to X, since dx/dt is constant:

$$t_1 = KX_1/S,$$

where X_1 is the vapor pressure of the internal alcohol required for death after time t_1. -K should then be directly proportional to P,

TABLE VI

From Figs. 6 and 7 there are obtained values of P at zero survival time, by extrapolation to the P axis. See text.

Age	P_0, mm.	
	f	m
days		
1	165.0	169.6
2	161.0	168.3
3	156.5	163.9
4	160.8	164.7
5	168.9	167.0
6	173.8	176.7
7	172.0	171.0
8	168.6	171.3
9	173.0	165.3
10	166.0	158.1
15	168.5	173.2
20	160.0	151.5
25	160.3	148.5
30	168.0	153.5
35	167.9	154.0
40	160.8	153.0
45	153.0	150.8
50	149.0	148.0

for each age and sex. It is apparent that since S is a constant for each age this will be formally correct, and will result in graphs exactly analogous to those in Figs. 6 and 7, the ordinate scale only being changed, as $KX_1 = St_1$. This means that X_1 varies with age in precisely the same way as S, and the curve of time-to-death at the minimum pressure of alcohol vapor which is lethal under the condi-

tions of these experiments must also exhibit the discontinuities shown in Fig. 11. From determination of the minimum lethal pressure for flies of each sex at one age it is possible to obtain by these considerations the values of X_1 at each age, and then from Figs. 6 and 7 to estimate the corresponding values of t_1. This has been done for flies 10 days old; X_1 was estimated to be $P = 10$ mm. for males.
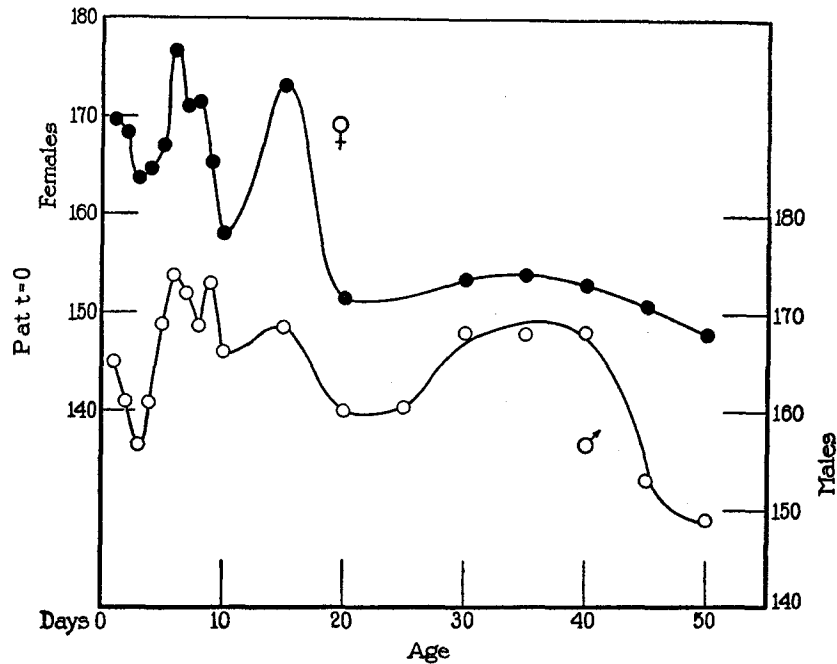

FIG. 10. The extrapolation of the lines in Figs. 6, 7 to $t = 0$, given as P_0 in Table VI, provides estimates of the pressure required to kill *Drosophila* instantaneously. See text.

The resulting curve of t_1 as a function of age is of the general character seen in Fig. 11, and is accordingly not reproduced.

It may be remarked that on the view that the vital resistance of the flies may be estimated in this manner under the foreshortening influence upon life duration of a markedly adverse condition, one should find a certain parallelism between the curves in Fig. 10 and the curve of average expectation of life as a function of age. In this sense we

agree with Teissier (1934 a) in regarding the estimates of expectation of survival (E_x) as suitable indices of viability. Such statistics are usually exhibited as calculated after a smoothing process has been applied to the raw data upon survival times. We shall have occasion to point out that in the case of *Drosophila* such a smoothing of the observations may be quite illegitimate and may lead to the suppression of information of considerable biological interest. The E_x curve for pure lines of *Drosophila*, from smoothed data, is a continuous declining curve. Teissier (1934 a) has given one E_x curve, however, computed from Gowen's data on triploids (referred to by Teissier as "intersexes") (Gowen, 1930–31), which shows cyclic fluctuations superimposed upon the downward trend. We shall shortly have occasion

TABLE VII

At the ages where resistance (P_0) goes through a maximum, R has a sharp change in curvature, a critical point (R_c).

	♀♀		♂♂	
	R_c	$Po_{max.}$	R_c	$Po_{max.}$
Age, days	35	35	37	36
	15	15	20	18
	9	8	9	9
	6	6	6	6

to see that the phenomenon is apparently a general one for inbred *Drosophila*.

Before leaving the curves in Fig. 10 for the present, we note that P_0 goes through a maximum at each age where the curve of $1/S$ exhibits a sharp change of curvature, and only at these ages, for each sex (Table VII). The correspondence cannot be accidental. It signifies that fluctuations in the invasibility of the fly for alcohol, determined by age, are correlated in a specific manner with changes in the rate of change of resistance to the inner toxic action of the alcohol. The first derivatives of P_0 and of S with respect to age show completely parallel behavior. Both must therefore be determined by the general course of more fundamental changes in the fly, of which the expression in various ways may be taken to give estimates of the progress of aging.

The existence of the complexities here revealed makes it plain why
the data upon time-to-death as a function of age, under constant con-
ditions of alcohol vapor pressure, cannot be fitted by one smooth
curve (*e.g.*, in Fig. 2). By means of the graphs in Figs. 6 and 7 esti-
mates may be obtained of the mean time-to-death at each age and at
constant alcohol vapor pressure, weighted by all the observations at
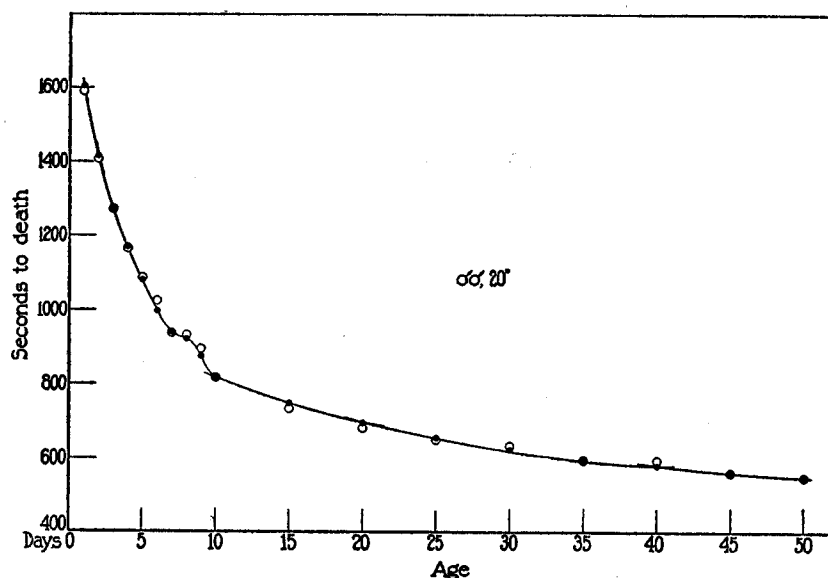each age.   In Fig. 11 such results are illustrated for ♂ ♂ flies at

FIG. 11. The solid circlets are durations of time-to-death as a function of age,
interpolated on the graphs of Fig. 7 at vapor pressure "25°C." The open circlets
are the actual determinations.   The solid circlets signify *calculated* times-to-death.
The discontinuities forbid passing a single smooth curve through the determina-
tions.

20°C. (*cf*. Fig. 2).   The discontinuities there shown are faithfully
reflected in the observations at this temperature (Tables I, IV).   They
result from the fact that the toxicity of alcohol and the ability of alco-
hol to penetrate the body of the fly are a complex function of age.
Similar curves are obtained at other values of *P*.   (The discontinuities
need not appear at precisely the ages corresponding to singular points
in Figs. 9 and 10.)

V

In connection with problems of the general type which here concern us it is customary to tabulate measures of the dispersions of the averages of observations. It cannot be said, however, that maximum use has always been made of such statistics, even in their bearing upon the question of curve fitting. When really homogeneous data are available, observed variation in the expression of an organic property may be fruitfully related to magnitudes of a known relevant variable; in this way the capacity of the organic system to exhibit variation of performance in the matter considered may be expressed quantitatively (cf. Crozier and Pincus, 1931–32, etc.; Crozier, 1929, 1935).

Three large categories of variableness contribute to the differences in time-to-death in the present experiments, under fixed conditions: fluctuation in the performance of the observer and of the apparatus; variations in the resistance of the flies to the toxic action of alcohol; and variations in the resistance of the flies to the penetration of alcohol.

When the standard deviations of the observations show a specific relationship to the values of an independent variable, the observer being the same, and if the conditions of observation are not materially influenced by the independent variable, one is safe in assuming that the variation indices are not a function of the process of observation,— or rather that their coefficient in terms of the independent variable is not such a function. In the present case the length of the time-to-death is the only variable directly connected with the process of observation. We assume that the flies have been correctly assorted as to sex, and that the variations connected with the introduction of the flies into the alcohol atmosphere are not significant. The maximum possible error in age of any one fly is $\pm$ 6 hours; variation introduced from this source would be largest in effect at the lowest ages; the fact is, however, that σ_t/t is lowest at these ages; hence variation from this cause is without recognizable effect. Since it turns out that σ_t is not a continuous function of t, there is no reason to suppose that t has, of itself and as connected with the process of observation, any direct influence upon σ_t. The times involved are never so short that the observer is hurried, and there is every evidence that the timing of one death does not influence the recorded time of the next observed death.

Careful examination of the properties of σ_t shows that the coefficient of variation, σ_t/t, as a function of age, exhibits the cusped character of the plot of R as a function of age. We have pointed out that R has the meaning of a *resistance*; it signifies the reciprocal of the change in time-to-death for a fixed increment in P, or, since the relationship is rectilinear, the change in P required to bring about at each age a given change in time-to-death. The relation between P and t shows that diffusion of alcohol into the fly is the significant process; the resistance to this diffusion must operate during the whole time up to the estab-
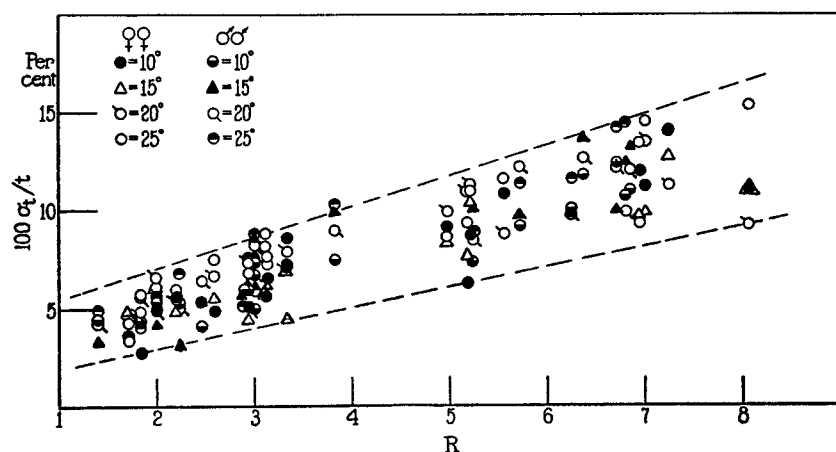
Fig. 12. Relative variation of time-to-death as a function of resistance to diffusion of alcohol. Data for individuals over 20 days old are not included at the two highest vapor pressures used (see Figs. 13, 14).

lishment of the lethal condition in the fly, hence the relative variation of t may be expected to be directly proportional to R. Fig. 12 shows that for $\male \male$ and for $\female \female$, indistinguishably, this is indeed the case. The observations at each value of P are plotted separately. At the two higher values of P, established at 30° and at 35° respectively, for flies over 20 days old, the value of R changes (Figs. 6, 7, etc.). These data are omitted from Fig. 12; in Figs. 13 and 14 it is demonstrated that for them the same kind of relationship obtains between σ_t/t and R, but that the proportionality factor is different, and also the behavior of the standard deviation of σ_t. The latter quantity is responsible for

the progressively wider scatter of σ_t/t at the higher values, when many tests of equal weights are available.

These considerations provide a rational statement of the capacity of this stock of *Drosophila* to exhibit variation in time-to-death due to
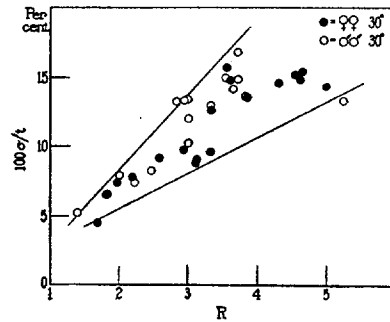

FIG. 13. Relative variation of time-to-death as a function of R at vapor pressure "30°C.," individuals above 20 days old.
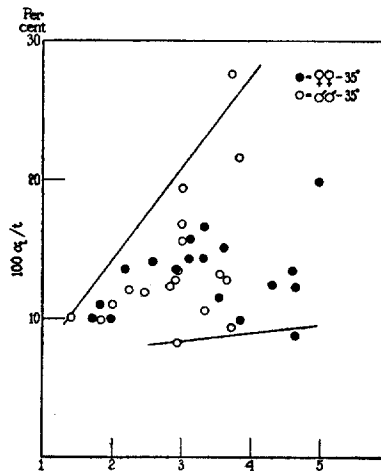

FIG. 14. Relative variation of time-to-death as a function of R at vapor pressure "35°C.," for individuals above 20 days old.

alcohol vapor. The formulation adds to the evidence that the diffusive invasion of the fly by alcohol is the only significant variable involved in the production of the lethal condition, aside from the minimum lethal internal dose, and that both are functions of age. The

specific differences between males and females do not concern the variation in time-to-death, but have to do only with differences in R and in lethal dose. Again there is no evidence, from the variation data, that protoplasmic reaction with the alcohol determines the shape of the P, t, curve. Conceivably, different stocks, or the effects
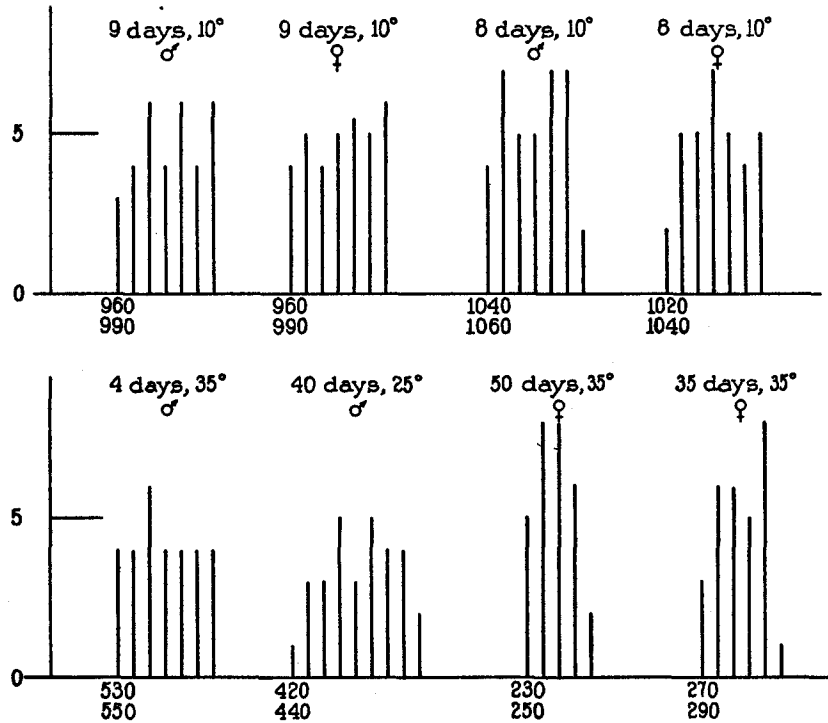

FIG. 15. Frequency distribution of time-to-death in groups of *Drosophila*. See text.

of different nutritive conditions, could be compared on this basis of the capacity to show variation in the time-to-death.

It has commonly been assumed that the susceptibilities of individuals in a homogeneous population are (or should be) distributed on a normal probability curve (*cf.* Clark, 1933). In the present material this is very far from being the case. The actual distributions are characteristically of the sort shown in Fig. 15 for 4 day old ♂ ♂ at 35°—flat distributions of a rather unusual type; they could result

from one of several causes into which we will not now inquire.[1] With older flies at the highest vapor pressure of alcohol the distribution of time-to-death is of the kind shown in Fig. 15 for 50 day old flies at "35°," again indicating that for the older flies, with high vapor pressure of alcohol, the variation of performance is of a different character. Presumably this is determined by physical breakdown of resisting surfaces under these conditions; these latter distributions show definite skewing toward the lower times-for-death.

VI

The periodic changes in resistance of *Drosophila* to penetration of alcohol from its vapor, and in the ideal vapor pressure required to kill instantly, call for interpretation. The explanation suggested is that in the life of the imago there occur periodic surges of metabolic activity similar to those associated with ecdysis. Prothetely indicates that moults can be experimentally suppressed. There is no real objection to assuming that in imaginal dipterons there may occur "moults" which fail to materialize. One expression of such processes would be assumed to be an alteration of the rate of "hardening" of the outer surface of the animal, including that of its tracheae and air sacs. The linings of air sacs and tracheae of house fly and honey bee apparently contain no chitin (van Wisselingh, 1924; Campbell, 1929), but in any case there is no reason to believe that the increasing toughness of the insect exoskeleton with advancing age is due to increasing deposition of chitin. The general increase in toughness with age is a well recognized property of the exoskeleton, but is due to the peripheral deposition of substances other than chitin (Campbell, 1929).

Periodic changes of the character suggested would be expected to affect diverse aspects of metabolic expression. The careful scrutiny

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[1] For example, it might well be supposed that at any one age there is a frequency distribution of penetrability with respect to alcohol vapor which is monomodal and essentially symmetrical, whereas the distribution of resistance in terms of toxicity of alcohol is heavily skewed in the sense that the mode is toward the low resistance side. There is much general evidence from other cases to support this notion. The variation in time-to-death would then be a product function of these two possibly independent variations; this could easily produce a very flat distribution.

of existing data upon *Drosophila* should reveal substantial indications of their reality, if they occur. The life duration tables for *Drosophila* are usually given after the observations on survival time and age have been smoothed. The most direct approach to these figures is to consider the raw death rates (d'_x) at each age. In Fig. 16 we have plotted the summated observed death rates $(\Sigma d'_x)$ given for Pearl's Line 107 (Pearl and Parker, 1924; Pearl, Parker, and Gonzales, 1923). It is apparent that there occur systematic deviations in the rate of
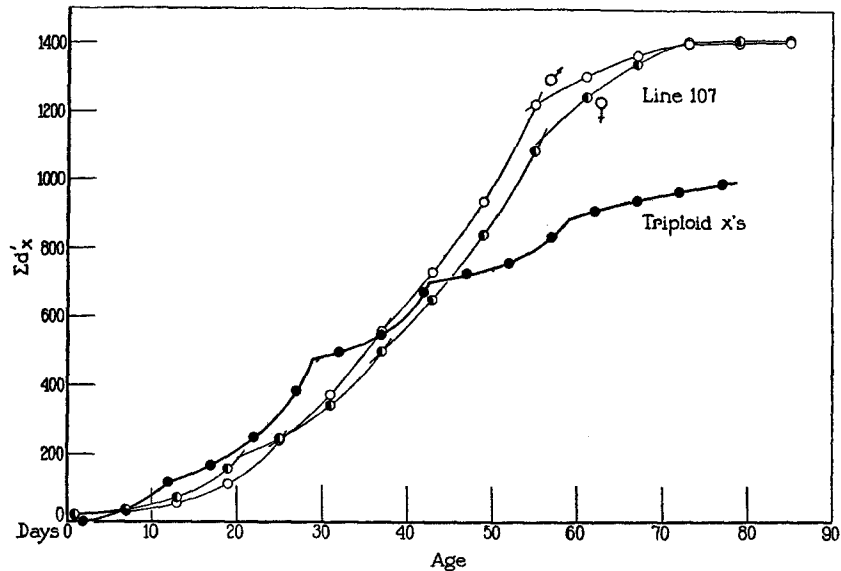

FIG. 16. Summated observed occurrences of death in male and female *Drosophila* of Line 107 (Pearl and Parker, 1924) and for triploid X individuals of Gowen (1930–31).

accumulation of deaths, which are in the same sense in the two sexes. This might possibly be the result of some element of the observational procedure, but the data on Vestigial show precisely the same sort of thing. A good test seems to be given by Gowen's (1930–31) tables; from his survivorship data, in which the type females and males show the effect already noted for Line 107 and Vestigial, plots are given in Fig. 16 and Fig. 17 of $\Sigma d'_x$ for his triploid females (3 X, 9 autosomes) and sex intergrades (2 X, 1 Y, 9 autosomes). We should expect an

accentuation of the irregularity of the $\Sigma d'_x$ curve in the triploid females, and in the group of unbalanced sex intergrades a statistical suppression of this effect, since the expression of intersexuality is variable. The curves show this to be the case, and appear to rule out the consideration of the discontinuities of rate of appearance of deaths as artifacts. When ♂♂ and ♀♀ data for a given stock are lumped, the discontinuities practically vanish. It is quite desirable that an analysis of such findings should be based upon daily observed death rates, but it

FIG. 17. Summated observed occurrences of death in unbalanced intersexed *Drosophila*, data of Gowen (1930–31).

is notable that in the several sets of observations available the locations of discontinuities in the first derivative of the $\Sigma d'_x$ line are specific.

The assumption basic to the customary treatment of survivorship curves and related functions implies that an individual death is the accidental outcome of a succession of accidents, and that the derivatives of the survival ratio are not discontinuous. Since in genetically homogeneous material the form of the survivorship curves is subject to inheritance, and is determined by the genetic constitution, under fixed conditions (Pearl and Parker, 1922; Pearl, Parker, and Gonzales, 1923; Gowen, 1930–31), the initial basis for such treatment seems not to exist. If the shape of the survivorship curve is a function of genetic

constitution, then with a genetically uniform population it is not permissible to smooth the data by a least squares procedure; with a sufficiently heterogeneous population the situation is quite different. It is apparent that any derived measures of mortality for *Drosophila*
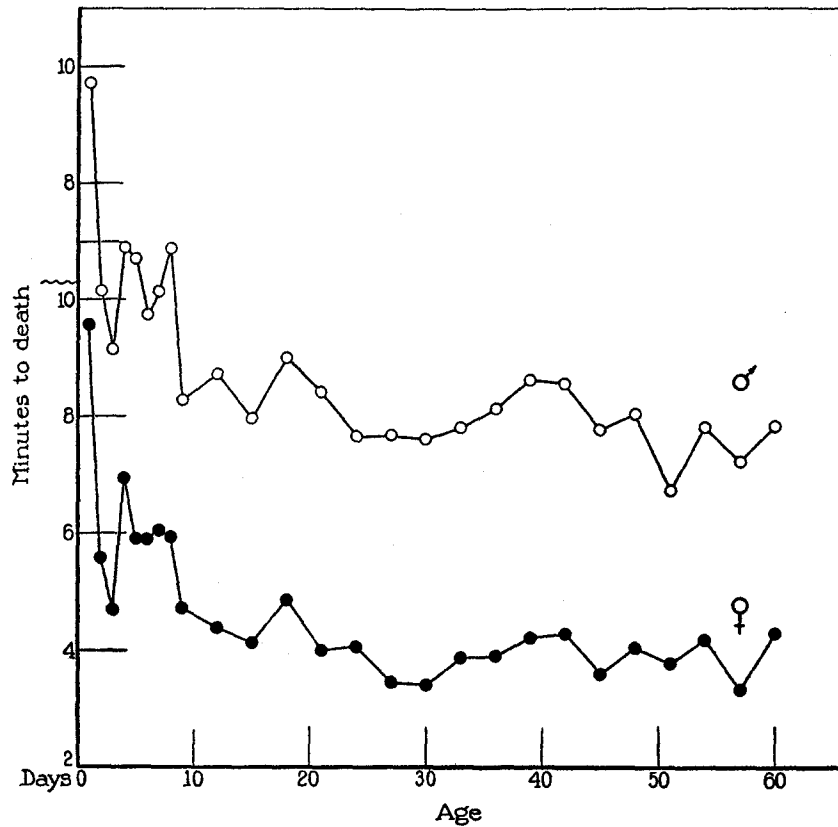

FIG. 18. Data from Pearl, White, and Miner (1929) for time to anesthesia in male and in female *Drosophila* of their Line 107. The discontinuities in change of curvature are discussed in the text.

must contain the irregularities exhibited in Figs. 16 and 17; consequently the interpretation of the "force of mortality" and related statistics, in this connection, must be recast; the outcome of experimental investigation of these matters must remain rather indefinite so

long as conclusions depend upon the comparison of artificially smoothed curves. It is suggested that in fact the likelihood of the occurrence of death is relatively greater, for *Drosophila*, at periods corresponding to the events which we have referred to as "suppressed moults;" these are supposed to have the character of critical metabolic periods. In dealing with larval insects it is a commonplace that deaths accumulate at periods of ecdysis.

We do not possess life table data for the strain of flies used in our experiments with alcohol, but we are led to expect that under the same conditions of culture such a table would reveal cusps at or a little after those ages which correspond to cusps in our curve of resistance to penetration of alcohol from its vapor (Figs. 6, 7), and to valleys in the curve of P_0 as a function of age (Fig. 10). Additional information may be obtained by a consideration of the lipoid content of *Drosophila* as a function of age, which we shall discuss subsequently. It may be possible to secure in this way a mechanism to account for the fact that the lethal internal amount of alcohol and the invasibility of the fly vary together.

In the case of Pearl's Line 107, however, a partial comparison can be instituted. Pearl, White, and Miner (1929) measured the time for anesthesia as a function of age, with one pressure of alcohol, in this line. The comparison is unsatisfactory, because we need to know the relation between time for anesthesia and vapor pressure of alcohol, at each age. But it is apparent that the data (Fig. 18) do indeed show consistent fluctuations in resistance to anesthesia, which are apparently valid and not unlike those fluctuations in the rate of incidence of dying which Fig. 16 reveals, and of a type entirely similar to what we have seen in Figs. 9 and 10. We are quite unable to accept the idea that a curve of the Gompertz type for force of mortality can be reasonably used to describe such observations, either theoretically (Pearl, White, and Miner, 1929; Teissier, 1934 b) or on any basis of expediency.

SUMMARY

The mean time-to-death (t) of imaginal *Drosophila* of an inbred line in alcohol vapor of constant partial pressure (P) is a declining rectilinear function of P for each age. The time-to-death depends upon the diffusion into the fly of an amount of alcohol sufficient to kill. It

does not depend upon any measurable property of a reaction between the substance of the fly and the alcohol which produces death. The relation between t and P is independent of temperature, but the invasion coefficient $S = -\Delta t/\Delta P$ declines with age and differs for the two sexes. The first derivative of S with respect to age exhibits sharp discontinuities. The internal alcohol required to kill declines with age, varying with S. The relative variation of t, σ_t/t, is directly proportional to the resistance to diffusive penetration of alcohol R, where $R = 1/S$.

The vapor pressure of alcohol estimated to kill instantaneously shows periodic fluctuations with age; these are precisely correlated with changes in the slope of S as a function of age.

Periodic fluctuations of invasibility by alcohol, and of the lethal dose, are interpreted as due to the incidence of suppressed moults. It is shown that in the accumulation of deaths as a function of time (age) in a genetically uniform population of Drosophila of one sex, similar fluctuations are apparent in the rate. The statistical smoothing of such data is not legitimate.

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