Table 2. Mean number of flies, Y, produced per week between weeks 16 to 68 by four experimental populations of *Drosophila serrata*, and regression coefficients, b, of the number of flies produced on time (weeks as time units) with t and P values for significance of regression.

Y	b	t	P
	Single strain at 25°C		
1434	+1.05	0.43	>.50
	Hybrid at 25°C		
1939	−2.08	0.64	>.50
	Single strain at 19°C		
795	+1.87	1.20	>.20
	Hybrid at 19°C		
1244	+0.69	0.30	>.50

ditions, natural selection is expected to produce and to maintain genetic constitutions which allow the populations to exploit maximally the available food sources. Any mutation arising in the experimental populations which would increase their ability to transform food into living matter had probably originated in the past and had been incorporated into the genetic endowment of the population. The second hypothesis is that competition for food during the immature stages was so strong that the maximum genetic improvement had already been achieved during the first three to five generations of the experiment, which are not included in the regression analysis. The higher productivity of the hybrid populations seems to support this hypothesis.

As for the adult flies, competition for space among them may not be so strong as competition for food among the larvae. On the other hand, it is unlikely that during their past history these populations have been living in their natural habitats with available space as limited as it is in the experimental environment. Adult crowding is a new environmental factor for these flies, and evolutionary adaptation is therefore more likely to occur. The genetic variability present in the populations, or arising by mutation and recombination, gives origin, under the action of natural selection, to new genotypes highly fit to survive under crowded conditions. In *The Origin of Species* Darwin wrote that natural selection "tends to the improvement of each creature in relation to its organic, and inorganic conditions of life." When drosophila flies are exposed to a novel condition of life, such as adult crowding, natural selection improves their genetic constitution in relation to that condition.

The conflict between adaptive fitness and genetic variability has been pointed out (*6*). The mutation process furnishes the raw materials from which adaptive changes are constructed, but it produces also a multitude of poorly adapted variants. Under the action of natural selection, those genetic variants in a population which increase the adaptation of the population to the environments in which it lives are preserved. Populations of the same species living in geographically widely separated regions are expected to have different genetic constitutions. Two such populations, one from New South Wales in Australia and the other from New Guinea, were used in these experiments to initiate hybrid populations. The hybrid populations may therefore carry larger amounts of genetic variability. The probability that highly adapted genotypes will be produced during exposure to new environments is greater in the hybrid than in the single-strain populations. Figures 1 and 2 show that the performance of the hybrid populations is superior for both the number of flies produced and the total population size. Similar results have been obtained in other cases (*3, 5, 7*). Moreover, Table 1 shows that the rate of increase in population size with time is considerably higher in the hybrid populations than in the single-strain populations. In other words, the hybrid populations are evolving faster. The rate of increase in the population size of the hybrid populations is approximately double that of the single-strain populations. The difference between the regression coefficients is statistically significant for flies maintained at 19°C ($t = 2.11$, $P < .05$), but not for flies maintained at 25°C ($t = 1.03$, $P > .20$).

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Collagen Defect Induced by Penicillamine

Abstract. *Collagen synthesis, as judged by the accumulation of collagen in a subcutaneous, induced granuloma, was significantly decreased by penicillamine. Penicillamine also caused a marked increase in the amount of soluble collagen in skin and a sharp drop in insoluble material. These findings, which reflect an abnormal pattern of collagen metabolism, are accompanied by an inhibition of wound-healing and by skin fragility.*

The resistance of penicillamine (β,β-dimethylcysteine) to metabolic degradation and its strong capacity to chelate metals suggested to Walsh (*1*) the possibility of using this compound in hepatolenticular degeneration (Wilson's disease), a disturbance accompanied by the accumulation of copper. It is presently under investigation for the treatment of macroglobulinemia, the cold-agglutinin syndrome, and in rheumatoid arthritis (*2*). It reduces the concentration of cystine in the plasma and urine of patients with cystinuria (*3*). The administration of penicillamine causes a number of side reactions which affect the connective tissue structures (*4*).

Subcutaneous granulomas were in-

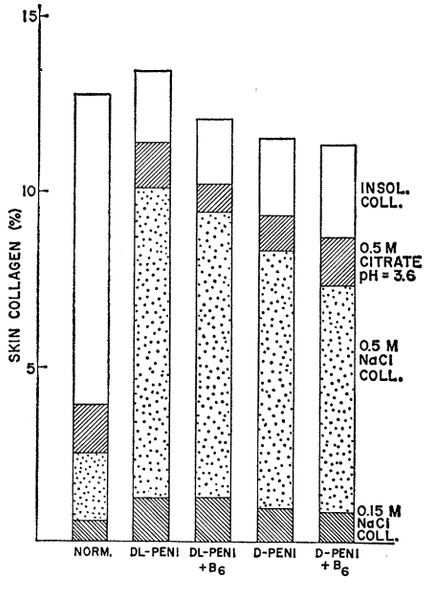

Fig. 1. Collagen distribution among the different soluble and insoluble fractions extracted from the skin of control rats and from those receiving DL or D-penicillamine, with or without additional supplements of pyridoxine. The height of the bars illustrates the percentage of each collagen fraction present in fresh skin.

Table 1. Supplements of pyridoxine (vitamin B_6) in the diet, dose of DL- or D-penicillamine, and average, daily body-weight increment over a 14-day period. There were six rats in each group.

Vitamin B_6 (mg/kg of diet)	Penicillamine (mg/day)		Avg. daily weight gain (gm)
	DL	D	
10	None	None	5.9
10	21		2.5
100	31		5.6
10		28	4.9
100		29	5.8

duced by implanting polyvinyl plastic sponges (weighing 100 ± 3 mg) under the skin of young rats of the Holtzman strain. The D- or DL-penicillamine was mixed with the diets in a proportion of 0.25 percent and the dose calculated was based upon the food intake of the rats. Wilson and du Vigneaud (*5*) found that L-penicillamine can induce a pyridoxine (vitamin B_6) deficiency, presumably owing to the formation of a thiazolidine complex (*6*), which makes this vitamin partially unavailable. Therefore, in order to overcome this effect, pyridoxine concentration in some instances was increased to 100 mg per kilogram of diet.

The pyridoxine supplement, the average DL- or D-penicillamine dose, and the daily gain in body weight over a 14-day period are summarized in Table 1. At the end of the experimental period the animals were killed and collagen at the site of the granuloma was measured. The excised sponge, surrounded by newly formed connective tissue, was sequentially extracted with 0.15*M* NaCl, 0.5*M* NaCl, and 0.5*M* citrate, *p*H 3.6, using a VirTis homogenizer. The remaining insoluble collagen was dissolved in 0.3*M* hot trichloroacetic acid. All fractions were dialyzed, hydrolyzed with acid, and analyzed for collagen by measuring hydroxyproline (*7*). A significant drop in the amount of insoluble collagen in the granuloma was observed in all animals receiving penicillamine (Table 2). The drop was most marked with DL-penicillamine and was accompanied by an increase in collagen that could be extracted with 0.5*M* NaCl. Analyses of the skin also showed that penicillamine caused a great accumulation of collagen in this same fraction (Fig. 1). Simultaneously, a compensatory decrease in the insoluble collagen occurred. This marked deviation from the normal pattern is of significance, since we know of no agent that will produce changes of such magnitude. Lathyrogenic compounds, such as β-aminopropionitrile, cause an accumulation of collagen precursors, probably by inducing a defect in collagen aggregation (*8*). However, the changes induced by lathyrogens are accompanied by severe growth impairment and are less than those provoked by penicillamine (*9*). In addition they fail to inhibit collagen synthesis around subcutaneous sponge implants (*10*), while penicillamine produces marked inhibition.

Table 2. Collagen content of the different soluble and insoluble fractions present in a 14-day-old subcutaneous granuloma removed from the rats described in Table 1. The values represent milligrams of collagen per total granuloma.

Soluble collagen (mg)			Insoluble collagen (mg)
0.15*M* NaCl	0.5*M* NaCl	0.5*M* citrate, *p*H 3.6	
	Group 1		
0.78	0.45*	0.76	13.8*
	Group 2		
.83	1.25*	.69	6.9*
	Group 3		
.95	1.05*	.89	8.7*
	Group 4		
.69	0.51	.74	9.5*
	Group 5		
.78	.50	.81	10.8*

* Statistically significant differences.

We repeated the foregoing experiments on another group of animals, including a pyridoxine-deficient group. These experiments confirmed our previous results and indicated that pyridoxine deficiency was not responsible for our observations since collagen distribution in the pyridoxine-deficient group did not differ appreciably from the controls.

In view of the marked effects on collagen, a study was made of the tensile strength of wounds as well as that of normal skin. Specimens, 5 mm wide, were tested for their tensile strength by means of an Instron machine, with the cross-head speed set at 5.1 cm/min. The force required to cause rupture is tabulated in Table 3. The rate of wound-healing was not dependent on the amounts of pyridoxine in the diet. DL-Penicillamine significantly inhibited wound-healing. In addition, penicillamine caused significant changes in the tensile strength of normal skin from a nonwounded dorsal site. The treated animals, especially those receiving the highest dose, showed a very pronounced decrease in strength. These findings in the rat support the observation (*4*) of the occurrence of increased skin friability at sites subject to pressure or trauma, such as knees, shoulders, elbows, and toes, and which lead in some instances to formation of hemorrhagic vesicles. Cysteine and methionine are associated with the process of wound-healing, the rate of which is directly proportional to the amount of sulfur-containing amino acids in the diet (*12*). Penicillamine may decrease the availability of cysteine by formation of a penicillamine-cysteine disulfide (*3*). Collagenase was shown to be irreversibly inhibited by cysteine. Also, metal-sequestering agents of the type *o*-phenanthroline and 8-hydroxyquinoline completely inhibit collagenase activity when added to the incubation media (*13*). Consequently a compound which antagonized cysteine (either by competitive antagonism or by decreasing its availability) should stimulate collagenolysis.

Table 3. Tensile strength of wound area and dorsal skin from normal, pyridoxine-deficient, and penicillamine-treated rats. Seven rats were tested in each group.

Vitamin B_6 (mg/kg of diet)	Penicillamine (mg/day)	Gain in body wt. (g/day)	Tensile strength (5-mm section)*	
			Wound	Skin
None		2.1	244	970
2.0		5.3	244	1224
10.0		5.5	226	1188
10.0	23	3.7	127†	281†
100.0	30	5.0	113†	163†

* Force in grams required to rupture skin.
† Statistically significant differences.

Because of the almost normal growth of the animals receiving penicillamine together with an adequate supplement of pyridoxine, it would seem that this disturbance is quite specifically related to collagen metabolism. Penicillamine may be a valuable tool to aid in further understanding of the process of collagen aggregation and breakdown. Our results are also a reflection of side reactions which accompany the use of penicillamine and the underlying mechanism of its action.

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Antibody-Complement Complexes

Abstract. *Soluble complexes containing the second and fourth components of guinea pig complement, as well as hemolytic rabbit antibody, have been prepared by elution from sheep erythrocytes carrying these factors. These complexes render erythrocytes susceptible to lysis by the other factors of guinea pig complement, without the usual requirements for hemolytic antibody, Ca++, and Mg++.*

In the present report we describe the preparation and some properties of a new kind of complement (*1–3*) intermediate which hemolyzes unsensitized sheep erythrocytes (*4*) in the presence of EDTA (*5*) if the factors of the C′3 group are supplied. Evidence is presented that this intermediate contains antibody, C′4, and C′2.

For the purpose of this investigation we prepared purified anti-sheep erythrocyte 7*S* antibody possessing low combining affinity (*6*). The 7*S* antibody was separated from rabbit antiserums to boiled sheep-erythrocyte stromata by chromatography on diethylaminoethyl cellulose. The fraction of the antibody population having low combining affinity was then selected by two successive absorptions with washed sheep erythrocytes at 0°C, each followed by elution of the low-affinity antibody fraction at 37°C. The eluted antibody was concentrated by precipitation with ammonium sulfate. The purified antibody was homogeneous as judged by chromatography on Sephadex G-200, analytical ultracentrifugation, and immunoelectrophoretic analysis. Its combination with sheep erythrocytes was completely reversible by dilution, or by shift of temperature. At a cell concentration of 7.7×10^7 per milliliter (1.06×10^5 receptors per cell) and 1.88 μg of antibody per milliliter, 51, 23, and 18 percent of the antibody was bound to the cells at 0°, 30°, and 37°C, respectively.

For preparation of the intermediate SAC′1a, 4, 2a (*7*), 1 ml of packed, washed erythrocyte stromata was treated with 0.5, 1, or 2 mg of purified antibody in a volume ranging from 1 to 10 ml in different preparations. $CaCl_2$ and $MgCl_2$ were added to concentrations of 0.15 and 1 m*M*, respectively. Two milliliters of ice-cold guinea pig serum were added and the reaction was held at 0°C for 10, 20, or 30 minutes. After centrifugal sedimentation, the stromata, presumably in the state StrAC′1a, 4, 2a, or beyond (*8*), were washed once with 0.5 ml of ice-cold M++-buffer (isotonic barbital-NaCl buffer, *p*H 7.3 to 7.4, ionic strength 0.147, containing 0.15 m*M* Ca^{++} and 1 m*M* Mg^{++} as well as 0.1 percent gelatin), and antibody-complement complexes (AC′C) were eluted by incubation for 5 or 10 minutes at 30°C in 15, 20, or 30 ml of M++-buffer containing approximately 500 units of partially purified guinea pig C′2 per milliliter. The inclusion of C′2 in the eluting buffer helped to maintain a steady state of SAC′1a, 4, 2a (*9*); this addition improved the yield, but it is not essential. The stromata were removed by centrifugation at 0°C, and the eluate containing AC′C was stored at −20°C.

The presence of AC′C in such eluates can be demonstrated by lysis of erythrocytes suspended in C′–EDTA (a dilution of guinea pig serum in buffer containing trisodium hydrogen ethylenediaminetetraacetate). Since formation of SAC′4, 2a is blocked under these conditions, the eluate presumably supplies AC′4, 2a, or an intermediate beyond this stage. The C′3 factors are furnished by the guinea pig serum. The possibility that the eluate contains a nonspecific lytic factor can be dismissed because there is no hemolysis without C′–EDTA.

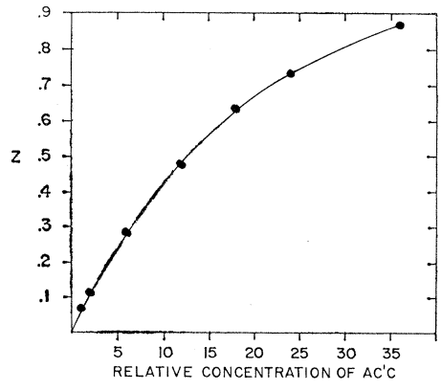

Fig. 1. Dose-response curve of AC′C. The ordinate, "*z*," representing the average number of membrane lesions per erythrocyte, equals the negative $\log_e$ of the fraction of surviving cells. Mixed equal volumes of 1/12.5 C′ in 20 m*M* EDTA buffer and E (3×10^8/ml) in M^{++}-buffer. One-milliliter portions of this mixture were treated with 1.5 ml of AC′C dilution and incubated at 30°C for 90 minutes.

The dose-response curve (Fig. 1) has a shape which fits the properties expected from AC′C, at least qualitatively. In accord with the one-hit theory of complement hemolysis (*10–12*), the curve is entirely concave to the abscissa; that is, its shape is not sigmoidal. The fact that it is not a straight line is to be expected because the antibody in AC′C was selected for low combining affinity, and therefore only part of the AC′C in the reaction mixture would be expected to be in combination with E, the remainder being in the fluid phase. In a companion experiment, not shown here, AC′C prepared with I^{125}-labeled antibody was used; direct proportionality was observed between radioactivity on the cells and the average number of holes produced in the cell membranes after

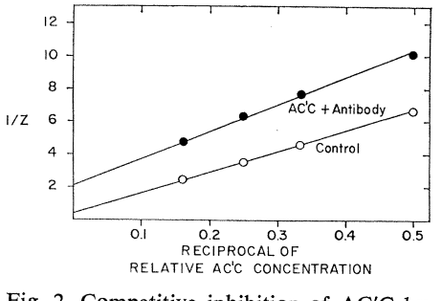

Fig. 2. Competitive inhibition of AC′C by free antibody. Experiment was set up as in Fig. 1, except that AC′C was mixed with purified hemolytic antibody. For control, AC′C was mixed with EDTA buffer.