centrifugation, using a 5 to 10 percent sucrose density gradient, gave more direct evidence that the inhibition was caused by components with a sedimentation coefficient smaller than 7S. Ten fractions were obtained from each tube, and inhibiting activity was recovered from top fractions of the split material, while corresponding fractions of control tubes with unsplit 7S γ-globulin contained no protein or inhibiting activity.

The β_{2A} myeloma protein was isolated from ten sera. Tests for purity were made by double diffusion tests in agar and showed that the preparations' contamination with 7S γ-globulin were less than 2 percent of the total protein content. All ten β_{2A}-globulins were clearly Gm(a—b—), whereas seven possessed the Inv(b) character. Five isolated 19S γ-globulins from macroglobulinemia sera were all Gm(a—b—), whereas two of them were Inv(b+). Finally, six Bence-Jones proteins were Gm(a—b—), whereas two of them possessed the Inv(b) character.

Since the 19S γ-globulins possessed the Inv(b) character, it seemed of interest to isolate these from individual normal sera to determine whether they were of the same type as the 7S γ-globulins. Euglobulin preparations were dissolved in glycine HCl buffer at *p*H 3.0 and subjected to density gradient ultracentrifugation. Selected fractions were carefully dialyzed against saline and tested immunologically for purity before Gm and Inv typing. Bottom fractions were obtained from six individuals with 19S γ-globulins free of 7S γ-globulin; the corresponding top fractions contained 7S γ-globulin and no 19S material. The Gm characters were present in the 7S fraction, whereas the fractions containing 19S γ-globulin all were Gm(a—b—). Two of the 7S γ-globulin fractions were Inv(a+b+) and four Inv(a—b+). Identical results were obtained in Inv typing of the fractions containing 19S γ-globulins.

It has previously been known that Gm and Inv determining sites are present on 7S γ-globulin molecules (*2*, *3*). Evidence has also been presented which indicates that 19S γ-globulins and β_{2A}-globulins lack the Gm characters (*7*). No evidence has been available concerning the presence of Inv determining sites on these proteins.

The present experiments confirmed that Gm determining sites are present only in 7S γ-globulins, whereas Inv determining sites are present also in 19S γ-globulins, β_{2A}-globulins, and Bence-Jones proteins. These findings and the results of the splitting experiments provide evidence for a common genetic makeup of a part of all four types of proteins. This is particularly interesting as this part of the 7S γ-globulin molecule is known to contain the antibody combining sites (*8*). The findings are in agreement with previous evidence from this laboratory (*9*) and elsewhere (*10*) which indicates that the immunological cross-reaction between 7S γ-globulin and the three other proteins is due to common antigenic determinants present on the S fragment after papain splitting of 7S γ-globulin, whereas antigenic determinants present on the F fragment appear not to be shared by the other proteins.

After papain splitting of 7S γ-globulin, the Gm determining site was found in one of the fragments only and the Inv site in the other. Irrespective of the precise action of papain on the 7S γ-globulin molecule, these findings indicate that the sites are present on different parts of the native molecule. The results with the highly homogeneous myeloma proteins are particularly relevant in this connection where the same myeloma protein contained the two sites in the different papain fragments. The molecules of 7S γ-globulin appears to consist of several polypeptide chains linked by disulfide bonds (*11*), and it is possible that the present findings correspond to the current concept of "one gene–one polypeptide chain" as exemplified by hemoglobin. However, this remains to be proved. At any rate, one gene, which also has the code of the Gm-determining site, might control the structure of one portion of the molecule. An independent gene may control the structure of another part of the molecule which contains the antibody combining sites (*8*) and also the Inv-determining site(s) (*12*).

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Relationship Between Temperature and the Metabolism of Experimental Ecosystems

Abstract. Short-term temperature variations of approximately 7°C above and below the normal maintenance temperature of balanced laboratory aquaria demonstrated that the metabolism of the ecosystem is practically independent of temperature. It is postulated that the closer a living system approaches the integration of a balanced ecosystem, the less it is affected by temperature.

The usual response to temperature of the metabolism of various single organisms is well known. However, the metabolic response of the balanced ecosystem, in which the food for the heterotrophs is made *in situ* by the community's autotrophs has not been studied frequently. Natural, balanced ecosystems are often large, not replicable, and temperature variation cannot be controlled. Such disadvantages may be overcome by using the microcosm method in which small replicate microecosystems are set up in the laboratory where environmental conditions can be more easily controlled than in the field. The microcosm method was used in this work.

The biota, water, and sediments for three benthic, fresh-water microecosystems studied were taken from the San Marcos River near the town of San Marcos, Texas, over a year previous to these experiments. By the time the measurements were made the communities had stabilized. The principal primary producers were *Vallisneria* and *Oedogonium*. Some of the other plants present were *Anabaena, Gleocystis, Merismopedia, Spirogyra, Cladophora, Chlamydomonas, Ankistrodesmus, Coelosphaerium, Chara, Achnanthes, Pinnularia,* and various very small green and blue-green unicellular forms. The animal contributing the greatest biomass to the microcosms was a lumbriculid oligochaete, *Sutroa* (?) whose habitat was in the sediments. The only other

macro animal was the snail *Physa.* Among the rather sparse microzoa were two species of rotifers, two species of nematodes, one ostracod species, a *Chlorohydra,* a copepod species, a hydrachnid species, and a turbellarian species. Among the protozoa, *Paramecium, Vorticella, Lacrymaria,* and *Difflugia* were occasionally encountered.

Thirteen sets of metabolic measurements were made on the three microecosystems designated 1B (three sets), 2D (six sets), and 3B (four sets). Each set of measurements consisted of four carbon dioxide diurnal rate of change curves (*1*). Changes in concentration of total carbon dioxide were followed by means of an improved *p*H-carbon dioxide method described by Beyers and Odum (*2*). This method involves the use of recording *p*H meters and the titration of samples of the microecosystem water with distilled water saturated with carbon dioxide to establish the relationship between *p*H and total carbon dioxide.

The controls of the constant-temperature room were not sufficiently exact to maintain a given temperature within an aquarium during the course of a day, nor to return that aquarium to the same average temperature after the temperature setting of the room had been changed. Therefore the average temperature during the day and the night of each diurnal rate-of-change curve was measured by integrating the temperature curve from a recording thermometer and dividing by the number of hours in the day or night. The first diurnal rate-of-change curve in the set was taken at a room setting of 23°C (nighttime mean 22.7°, extremes 19.4° to 22.4°; daytime mean, 23.7°, extremes 21.0° to 24.7°). The next curve was taken 24 hours after the end of the first curve. For this second curve, the setting of the constant-temperature room was 33°C (nighttime mean 30.1°, extremes 28.0° to 31.7°; daytime mean 30.9°; extremes 29.0° to 31.9°). Upon completion of the second curve the microecosystems were returned to their normal temperature for 24 hours. The room was then lowered by 10°C. The third diurnal metabolic measurement was made at this time (nighttime mean 16.9°, extremes 14.8° to 18.3°; daytime mean 17.7°, extremes 16.8° to 19.1°). After another 24-hour rest period at normal temperature the last carbon dioxide diurnal rate-of-change curve in the set was measured, again at normal temperature settings (nighttime mean 22.9°, extremes 21.2° to 24.0°; daytime mean 23.6°, extremes 21.4° to 24.8°). Within the set the four measurements were designated "normal before," "high," "low," and "normal after."

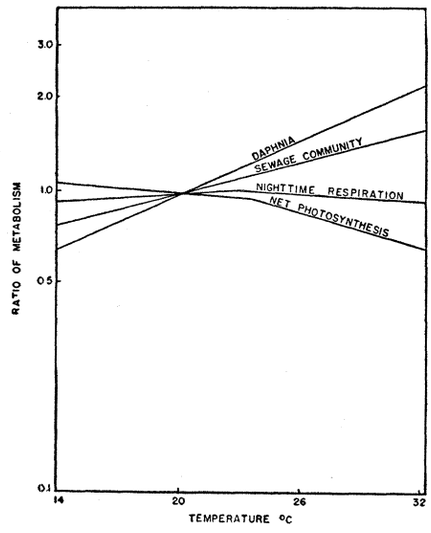

Fig. 1. Effect of temperature upon the metabolism of a single organism, a sewage community, and the nighttime respiration and net photosynthesis of four fresh-water laboratory microecosystems. All measurements of metabolism are plotted as the ratio of metabolism at a given temperature to the metabolism of the same organism or system at 20°C.

Between each measurement the temperature was returned to normal in an attempt to keep the temperature variation necessary to establish the conditions for one metabolic determination from affecting the next determination in the series.

Each group of curves yielded four measurements of total nighttime respirations and four measurements of total net photosynthesis: one measurement at high temperature, one at low, and two at the normal adapted temperature (Table 1). The term "total nighttime respiration" refers to the amount of carbon dioxide released per square meter of microcosm surface per 12-hour dark period, and "total net photosynthesis" refers to the amount of carbon dioxide taken up per square meter per 12-hour daytime period. This last figure is uncorrected for daytime respiration. All 52 sets of nighttime respiration data were averaged (Table 1) and the four average figures were plotted on semilogarithmic paper; the resulting nighttime respiration curves are shown in Fig. 1. The data on net photosynthesis were treated similarly.

The thermal independence of microecosystem metabolism is graphically demonstrated by Fig. 1, although the data show a slight tendency toward peak metabolism at the adapted temperature. The effects of temperature on the respiration of a single animal, *Daphnia* (*3*) and on the metabolism of a stream sewage community (*4*) are also shown in Fig. 1. To facilitate comparison of data originally presented in different units, the various metabolisms in the figure are plotted on the vertical axis as the ratio of metabolism at a given temperature to the metabolism at 20°C.

Since the steepness of the slope of the lines in Fig. 1 may be taken as a relative measure of the temperature dependency, it can be seen that the respiration of the single organism is more dependent on temperature than that of the sewage community, which in turn,

Table 1. Nighttime respiration (R) and net photosynthesis (P) for 13 experimental elevations and depressions of microecosystem temperatures. All data are expressed as grams of carbon dioxide absorbed or excreted per square meter per 12 hours.

Microcosm	Normal	Before	High		Low		Normal	After
	R	P	R	P	R	P	R	P
1B	1.14	1.29	1.33	0.88	1.01	1.14	1.27	1.48
1B	1.25	1.54	1.32	0.94	1.01	1.14	1.11	1.54
1B	1.09	1.52	1.57	1.31	1.31	1.80	1.34	1.66
Mean	1.16	1.45	1.40	1.04	1.11	1.36	1.24	1.56
2D	1.39	1.42	1.17	0.57	1.33	1.50	1.35	1.18
2D	1.19	1.88	1.72	1.64	0.85	1.85	1.54	2.29
2D	1.96	2.86	1.32	0.94	1.01	1.11	1.11	1.54
2D	1.67	1.85	1.27	0.91	1.20	1.71	1.51	1.51
2D	2.28	2.20	1.55	1.22	1.85	2.27	2.29	2.21
2D	1.48	1.51	1.83	1.61	1.41	1.39	1.94	1.97
Mean	1.66	1.95	1.48	1.15	1.28	1.63	1.62	1.78
3B	1.99	1.69	1.20	1.15	2.69	3.51	1.58	1.51
3B	1.54	1.32	1.58	1.56	1.25	1.38	1.42	1.48
3B	1.49	1.42	1.29	1.32	2.59	3.71	1.64	1.30
3B	1.41	1.34	1.10	0.84	0.83	0.80	1.04	0.92
Mean	1.61	1.44	1.29	1.20	1.84	2.35	1.42	1.30
Grand mean	1.53	1.68	1.40	1.13	1.41	1.79	1.47	1.58

is more dependent on temperature than the nighttime respiration of the balanced microecosystem. Since daytime respiration is not measurable, its similarity or dissimilarity to nighttime respiration can only be assumed. If one assumes that daytime respiration, unlike nighttime respiration, increases with increase in temperature, then the drop in net photosynthesis at high temperature may be explained as reflecting an increase in daytime respiration. However, if daytime respiration is assumed to be independent of temperature, then the decrease in net photosynthesis at high temperature must be ascribed to some inhibitory effect of high temperature. In any event, the effect is not great, and photosynthesis certainly is not stimulated by increase in temperature. A similar temperature relationship was found by Golueke (*5*) in the net photosynthesis of a planktonic community consisting of *Chlorella, Scenedesmus,* and bacteria.

The results of the present work suggest that the more a system approaches the integration of a balanced ecosystem, the less its respiration is affected by temperature. Such a hypothesis could be readily explained by postulating that within a highly integrated community, where the physiological and geochemical cycles have evolved an almost complete interdependence, the multiplicity of metabolic pathways assures the cyclic flow of energy and material regardless of the temporary closing of one or several pathways by temperature extremes. In a single organism, or in a group of organisms which are less well adapted to each other and to their environment, the fewer pathways may be more easily affected by temperature (*6*).

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Persistent Fear Responses in Rhesus Monkeys to the Optical Stimulus of "Looming"

Abstract. The approach of an object corresponds with a spatiotemporal optical stimulus consisting of a symmetrical expansion of a closed contour in the field of view. The visual equivalent of impending collision was isolated and compared with its sequential inversion. Infant and adult rhesus monkeys manifested persistent avoidance responses to "looming" but not to the inverse. This visual stimulus alone is a strong exciter of avoidance, and the response appears early in life.

The ability to avoid biologically dangerous situations is of ultimate importance to the survival of animals. The avoidance of dangers implies the ability to discriminate between stimuli signifying potential injury and stimuli signifying harmless or beneficial situations. Such stimuli are often optical, and they involve distance perception.

Walk, Gibson, and Tighe (*1*) investigated the "higher-order" visual stimulus reaching the ocular system from a drop-off of the ground—a danger for terrestrial animals. They used a "visual cliff" providing the optical input arising from a falling-off place. Avoidance of the cliff was studied with dark-reared and light-reared rats, and later studies by Gibson and Walk included other animals and human infants (*2*).

Our study is one of a series of cross-species investigations aimed at discovering visual stimuli sufficient for initiating avoidance and escape responses in animals at a given stage of development. The stimuli used are abstractions of those found in a natural environment.

The rapid approach of a solid body is also a natural source of danger for most animals. The optical stimulus arising from the approach of, or approach to, a body indicates an impending collision. One of us (J.G.) has previously proposed that the expansion of a closed contour in the field of view is specific to relative approach (*3*). Symmetrical expansion of any silhouette means a collision course, and when magnification comes to fill the entire 180° frontal field of view, a collision occurs. This optical stimulus may be called "looming." It is hypothesized to be a spatiotemporal stimulus, characterizing *any* case of impending collision with an environmental object. The mathematical properties of this stimulus have been described in recent studies relating to the prediction of time-to-collision by a human observer (*4*).

We have tested the effectiveness of this stimulus with rhesus monkeys, using an apparatus designed to provide the optical equivalent of an impending collision. A silhouette was made to undergo magnification or the reverse. A 25-watt, concentrated-arc, point-source lamp at the end of a 3-foot track projects the shadow of an object moving along the track onto a 6-foot square translucent screen. As the object is moved along the track perpendicular to the screen by an electric motor, the shadow expands or contracts geometrically.

In the particular study we are describing, the shadow was cast by a 1⅛-inch rubber ball. This resulted in a visual experience, for a human observer, of a dark circular object approaching or receding in a large luminous field at a constant high rate of speed. It was clearly a three-dimensional perception (*5*), not an experience in two dimensions. For purposes of control, this could be compared with a simple lightening or darkening of the screen produced by raising or lowering a shutter just in front of the lamp. This did not yield a three-dimensional perception for a human observer. The measured brightness of the unshadowed portion of the screen was 0.85 ft-lam, and the brightness of the shadow was 0.035 ft-lam, a ratio of about 24 : 1. The animal's cage faced the screen at a position 5 feet in front of it, the lamp being 5 feet behind it. The animals were observed from behind curtains on either side of the screen. The room was dimly illuminated.

Twenty-three monkeys, including eight infants 5 to 8 months of age and 15 adolescent or adult animals, were used (*6*). The trials were spaced approximately 45 seconds apart. The stimulus-event was produced only when the animal was facing the screen. Two hidden observers independently judged the animals' behavior as "abrupt retreat," or one of several other categories of response.

The four stimuli used were expansion of circle, contraction of circle, darkening of screen and lightening of screen. The conditions are sequential inversions of each other. The four conditions were given in a counterbalanced order to four animals on successive days, and one order each to the remainder of the animals.

It was found that the observers' judgments were in agreement in 98 percent of the trials. Responses were counted only when there was agreement.

The four stimuli resulted in two