eleven staff members are thus on leave, twenty others are giving full time to defense research in our own laboratories, and over forty more are devoting part time in connection with various advisory committees, as consultants to the Army and Navy, and in similar capacities. Much of the work in our own laboratories is carried on under contract with governmental agencies, the Institution being reimbursed for its added expenses in carrying out the work, but not for the regular expenses of the laboratory, the overhead or the salaries of scientists on the staff. This has allowed a greater expansion of the work than would have been possible had the Institution made the entire contribution. The heaviest burden has fallen on the Department of Terrestrial Magnetism, where nearly all the research is now on governmental problems, and the number of men in that laboratory in all categories has been more than doubled in order to carry it on. The Geophysical Laboratory, the Mount Wilson Observatory and the Nutrition Laboratory also have important efforts under way. The amount of the government funds made available for the added expenses of those programs has now reached nearly two thirds of the normal budget of the Institution.

This type of work is now being done in scientific laboratories all over the country; it was concentrated at first, of necessity, in some of the larger institutions, but gradually it is reaching out to others. The whole program is being enlarged to meet increased needs, especially in the field of medical research.

In order that the combined effort may be properly integrated, the President of the United States has, by Executive Order, created the Office of Scientific Research and Development as a part of the Office for Emergency Management, and has given this Office the task of coordinating all defense research, and, through its subordinate bodies, of supplementing the research of the Army and Navy in the development of instrumentalities of warfare and in medical research connected with defense. The president of the Institution is director of this office, and many staff members are members of its organization. Its main offices are located in the Administration Building of the Institution, under an arrangement whereby space, furnished at first to the government at nominal cost, is now made available for government purposes without charge. The chairman of the National Defense Research Committee, which is a part of the office, is now President James B. Conant, of Harvard University, and the chairman of its Committee on Medical Research is Dr. A. Newton Richards, of the University of Pennsylvania. The office has close relations with all governmental agencies and private organizations concerned with defense research. It has especially close relations with the National Academy of Sciences, which is the advisory body to all governmental agencies on their scientific programs, and of which our fellow trustee, Dr. Frank B. Jewett, is president; and with the National Research Council, with which many of our staff are associated, the important Medical Division being headed by Dr. Lewis H. Weed, of the Board of Trustees.

Many of the dislocations and stresses produced by the emergency are not pleasant. There is, however, one decidedly pleasant aspect of the matter. Owing to the close connection of the Institution with the defense research effort there is a continual succession of visits to the headquarters of the Institution from the outstanding scientists of the country, from members of our own staff from a distance, and from the many trustees of the Institution who are concerned with various aspects of the defense problem. When the emergency is over the scientists of the country will be better acquainted with one another, and they will also be better acquainted with the Institution. This should certainly be of a real benefit when our full normal program again occupies our laboratories, and when our aid and collaboration again become fully extended to those in other institutions whose research is closely allied to our own.

SPECIAL ARTICLES

A BASIC MECHANISM IN THE BIOLOGICAL EFFECTS OF TEMPERATURE, PRESSURE AND NARCOTICS

AN important property of isolated proteins and enzymes, heretofore not adequately recognized in studies on living systems, is that of reversible denaturation. Active native trypsin has been shown to exist in equilibrium with an inactive, denatured form.[1] The equilibrium may be influenced in diverse ways, *e.g.*, temperature, acid, alkali, alcohol. Evidence that such a reversible denaturation (RD) might be of major importance in controlling the intensity of bacterial luminescence led us to undertake a re-evaluation of certain factors—temperature, pressure and narcotics—in biological systems.

For this problem the luminescence of bacteria is ideally suited. Under given conditions, the intensity of luminecsence is proportional to the reaction velocity of the light-emitting enzyme, luciferase. Luminescence thus provides a unique and instantaneous measure of enzyme action within the living cell.

The influence of temperature on the luminescence

[1] M. L. Anson and A. E. Mirsky, *Jour. Gen. Physiol.*, 17: 393, 1934.

intensity of two unrelated species of bacteria is shown in Fig. 1. *Photobacterium phosphoreum* is a marine,

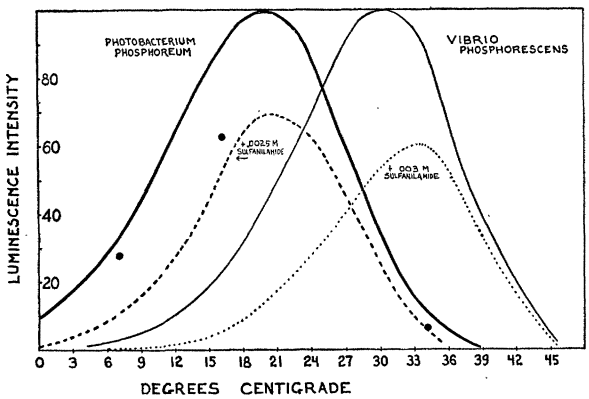

FIG. 1. The relation between temperature and luminescence intensity in suspensions of two, unrelated species of luminous bacteria, *Photobacterium phosphoreum*, (heavy line) and *Vibrio phosphorescens* (lighter line). The lines represent smoothed curves through a multitude of experimental points. The effects of moderately inhibitory concentrations of sulfanilamide are represented by the dotted lines. The solid points indicate the reversibility of heat diminution of luminescence intensity of *P. phosphoreum* when a normal suspenson is quickly warmed to 34° C., cooled at once to 16° and then to 7° C.

psychrophilic coccus, while *Vibrio phosphorescens* is a fresh-water, mesophilic vibrio. The essential similarity between the curves is quite apparent. The important difference is that each species has its own particular position on the temperature scale, with optimum temperatures of approximately 20° and 30°, respectively. These data clearly indicate that any factor whose influence on luminescence is affected by temperature, whether a chemical factor such as sulfanilamide (see Fig. 1)* or a physical agent (see below), must be considered in relation to the temperature characteristics of the particular species involved. Only in this way can one expect to obtain comparable results, whatever the species, or its particular range of temperature.

In regard to the temperature-intensity curves, the basic observation is that the decline in intensity above the optimum is almost entirely reversible on cooling, provided the high temperature is not maintained too long. This reversibility, previously unreported except for temperatures below the optimum, shows that neither a thermal destruction of the enzyme nor a killing of the cells is responsible for the decrease in luminescence intensity. The reversible reaction, however, does have a high temperature coefficient. The μ value of about 55,000 calories approaches the 67,600 calories reported by Anson and Mirsky[1] for the heat of reaction in the equilibrium between active native and inactive denatured trypsin. Other protein denaturations possessing temperature characteristics of the same order have been considered in relation to the theory of absolute reaction rates by Eyring and Stearn.[2] These facts strongly suggest that the decrease in luminescence intensity at higher temperatures is caused by a reversible denaturation of the enzyme, in this instance, luciferase (Harvey).[3,4]

The real key to an understanding of the intensity-temperature relation derives from the observation that hydrostatic pressure inhibits the reversible denaturation and thereby prevents the decrease in luminescence intensity which, at atmospheric pressure, occurs at high temperatures (Fig. 2).

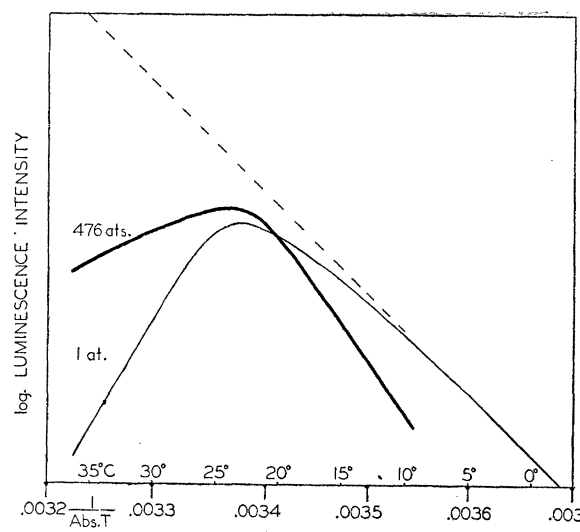

FIG. 2. The relation between temperature and luminescence at atmospheric and 476 atmospheres pressure. The extrapolated broken line shows luminescence when corrected for the reversible denaturation.

On the basis of these results, the control of luminescence may be diagrammed as follows:

$$A_n \xrightarrow{\mu = 17{,}000} A_n^* \longrightarrow A_n + h\nu \qquad \text{I}$$

$$A_n \xrightarrow{\mu = 55{,}000} A_d \qquad \text{II}$$

wherein native luciferase, A_n, becomes excited to A_n^* in an oxidative reaction, and then emits light in a reaction independent of temperature. The oxidative

* The sulfanilamide studies have been aided, in part, by a grant to one of us (F. H. J.) from the Penrose Fund of the American Philosophical Society.

[2] H. Eyring and A. E. Stearn, *Chem. Rev.*, 24: 253, 1939.

[3] E. N. Harvey, *Erg. d. Enzymfors.*, 4: 365, 1935.

[4] E. N. Harvey, *Ann. Rev. Biochem.*, 10: 531, 1941.

reaction (I) has an energy of activation of about 17,000 calories. The amount of active, native luciferase, however, depends on the equilibrium (II) between the native and denatured forms. This equilibrium, the reversible denaturation, has a heat of reaction of about 55,000 calories. Thus, at any temperature the actual light intensity is the net result of two simultaneous reactions: (I), the primary enzyme reaction, leading to light emission, and (II), an equilibrium governing the amount of active enzyme. At temperatures low with respect to the optimum, (I) predominates and (II) is negligible. At the relatively high temperatures, (II) predominates. At either extremity, the temperature-intensity curve (Fig. 2) is practically a straight line. At the optimum, (I) and (II) balance.

The action of pressure throughout all of the temperature range (Fig. 2) may be readily interpreted in terms of the foregoing scheme. Pressure is known to retard any reaction involving an increase in volume, such as that of a molecule in going from the normal to an activated state, or as in the process of denaturation, from the initial to the final state.[5] On this basis the pressure effects become understandable and can be related to physical chemistry. At the lower temperatures (Fig. 1, below 9° for *P. phosphoreum,* and below 19° for *V. phosphorescens*) pressure reduces the light intensity by slowing the primary enzyme reaction (I) that leads to luminescence. This pressure effect, of course, is assumed to be on the process of activation, not on that of excitation directly. At temperatures above the optimum, where the reversible denaturation predominates in controlling luminescence, the effect of pressure is to increase luminescence by shifting the equilibrium (II) to the left, thereby increasing the amount of active enzyme. At the optimum temperature, pressure affects (I) and (II) to approximately the same extent, with the result that very little change in luminescence intensity is observed. It can be shown that each of these effects of pressure conforms to the Glasstone-Laidler-Eyring[5] theory of absolute reaction rates sufficiently to permit the calculation of the temperature intensity relation at any pressure. This fact, in itself, constitutes the strongest evidence that these intracellular reactions obey the same laws that govern the reaction velocity in chemical reactions generally, and it leaves little doubt as to the correctness of the interpretations and assumptions involved.

The recognition of a reversible thermal denaturation as a major factor in the temperature-velocity relation raises several points of interest. Thus, the temperature setting of the two species of bacteria may be accounted for, both in regard to the different optimum temperatures and the general form of the curves. Such differences would merely indicate that the RD reaction becomes appreciable in each species at a different critical temperature; higher for some, lower for others. Basically, such specific differences, it would seem, must be genetic; but in a given organism, any factor which would influence the RD reaction or equilibrium would tend to shift the optimum temperature, as well as to affect the absolute intensity and the temperature coefficient. There are numerous biological phenomena which might be considered from this point of view.

In the majority of biological reactions the rate or intensity of a given process agrees with the Arrhenius relation for the velocity of a chemical reaction over a limited but clearly recognizable range of temperatures. With some justification this agreement has been interpreted as evidence that the course of events is under the control of a particular chemical reaction, having a definite temperature coefficient or μ value. Deviations, prone to occur as the temperature is raised toward the optimum, have been variously interpreted. Prominent among the explanations that have been advanced is the theory of Crozier. This theory postulates that a series of consecutive reactions, each with a different temperature characteristic or μ value, is involved in any biological process. According to their μ values, different reactions in the series have been thought to become the pace-setting, or "master reaction" at different temperatures. Deviations or "breaks" in the straight-line regions of the Arrhenius plot are presumed to represent points at which different reactions in the series assume control over the velocity of the process. According to the views expressed in the present paper, however, such "breaks" would represent the point at which the RD reaction, or equilibrium, becomes significant. In contrast to the Crozier theory, this is a simultaneous reaction, involving the same molecule, rather than a consecutive member of a series of reactions. Furthermore, the RD has a high temperature coefficient, whose true value is revealed only at temperatures beyond the optimum, or by introducing another variable, namely, pressure. Indeed, from the pressure-temperature-intensity data, it can be shown that, were it not for the RD equilibrium, the logarithm of luminescence intensity plotted against 1/T would constitute a straight line, without a break, for a considerable range beyond the normal optimum (dotted line in Fig. 2).

Since alcohol and certain other substances are known to influence the RD equilibrium of isolated proteins it is interesting to consider the possibility that the RD reaction might be the fundamental basis

[5] Glasstone, Laidler and Eyring, "The Theory of Absolute Reaction Rates." New York: McGraw-Hill, 1941.

of narcosis by such substances in the living cell. At the optimum temperature, pressure has little effect on the luminescence intensity of normal suspensions. If this intensity is first reduced by the addition of any of a series of lipoid soluble narcotics, pressure will largely or completely abolish the inhibition. Compounds included in this group are ether, alcohol, chloroform, ethyl carbamate, phenyl carbamate and novocaine. According to the views already expressed, these substances must cause a shift in the RD equilibrium, leading to the increased volume of the denatured molecule, reversible by pressure. Another group of narcotics decreases luminescence in a manner that is not reversible by pressure, although the inhibition is largely abolished by removing the substance from the suspension medium, or in certain cases by temperature. Barbiturates, chloral hydrate, sulfanilamide and p-aminobenzoic acid belong in this latter group, which must act by a different process, perhaps a chemical or adsorptive combination with the enzyme. Members of both groups, *e.g.,* urethane and sulfanilamide, have been found to act on the enzyme, without affecting the total luminescence, of extracted *Cypridina* luciferase and purified luciferin.[6] The significant point is that pressure effects make it possible to identify the probable action of the first group, in spite of the diversity of the chemical structure, as affecting the reversible denaturation of the enzyme, and to distinguish this from the action of another large group of narcotics.

Although the foregoing discussion has been restricted primarily to bacterial luminescence, it should be emphasized that the principles developed apply equally well to the energy exchanges of muscle. Here it can also be shown that the RD reaction determines the augmentation in tension which is obtained when pressure is applied at temperatures above the optimum, whether in muscles from tropical or temperate animals. Deviations from the Arrhenius relation may be shown to depend on the interference of a reversible denaturation with a high μ value. For this reason we would expect to find the RD processes conditioning the effects of temperature, pressure and chemical agents generally. The further delimitation of this type of reaction would seem, therefore, to be of the utmost importance.

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[6] F. H. Johnson and A. M. Chase, *Jour. Cell. Comp. Physiol.,* in press.

SUCCESSFUL REARING OF A SECOND GENERATION OF MICE ON AN ARTIFICIAL DIET

IN view of the known difficulty in raising mice on highly artificial rations we offer this preliminary report of the rearing of a second generation of this species on such a diet. The basal ration, as used, consisted of Labco casein, cerulose, crisco, Osborne and Mendel salt mixture with added zinc carbonate, agar, wheat germ oil and 3 per cent. rice polish filtrate factor II. This basal mixture was supplemented with cod liver oil concentrate, thiamine, riboflavin, pyridoxine, nicotinic acid, calcium pantothenate, choline, para-amino benzoic acid and inositol.[1] During the past month, linoleic acid has been added. The actual proportions of components used in the above diet (Diet A–2) as worked out by McElroy will be published later.

A group of 14 strain C_{57} black mice,[2] 5 males and 9 females, were placed on Diet A–2 at weaning. Of these 14, all 5 males and all but 2 of the females are alive and well after 184 days of subsistence on this diet. Growth curves of the whole group and their descendants in addition to the history of 10 of the original animals will be reported later, as not all the females were allowed to mate as soon as they were mature. Three females (Nos. 9, 8 and 12) were mated with a sibling male initially. To date female 9 has had 3 litters with a total of 10 live and 4 dead young. She did not lactate until the third litter was born on the 157th day of subsistence on the diet. Of this third litter of 4, she has successfully raised to weaning 3 young.

As a result of the first mating females 8 and 12 had litters totalling 10 live and 1 dead young on the 55th day on the diet. Both females lactated and 8 of the young (f_1) were raised to weaning and placed on Diet A–2; one young male died subsequently from an accident. At 69 days of age the 4 young females (f_1) were allowed to mate with two male siblings. All these females became pregnant readily, but one died of peritonitis 9 days after a vaginal plug was found. When 91 days of age, the 3 remaining females had litters totalling 16 live and no dead young. One of the young from each litter died during the first day of life. Although these were first litters, all three mothers lactated. Lactation gradually ceased in 1 mother and her 3 young were dead by the 13th day. The other 2 mothers reared their litters of 4 and 6 young successfully to weaning at 21 days of age. These 10 second generation (f_2) mice are now alive and growing well on Diet A–2 at 41 days of age.

The two original females (Nos. 8 and 12) have

[1] We are indebted to Merck and Company for supplying in pure form the various members of the vitamin B complex used in these experiments.

[2] Supplied by Dr. L. C. Strong.