0.5 ml homogenate in total volume of 1.0 ml were incubated in a water bath at 30° C. Each tube contained besides the homogenate: tris(hydroxymethyl)-aminomethane-HCl buffer pH 8.0, 0.10 *M*; $MgCl_2$, 0.01 *M*; sugar, 0.01 *M*; and ATP, 0.03 *M*. Immediately after addition of homogenate and at short intervals thereafter 0.1-ml samples were taken and treated with $Ba(OH)_2$ and $ZnSO_4$ and centrifuged. In the supernatant the reducing capacity was measured according to the method of Somogyi (*9*). Figure 1 shows the rapid decrease of the reducing capacity in the samples containing glucose and 3-methylglucose in the presence of intestinal mucosa homogenate and ATP. The reducing capacity did not decrease in either of the sugars studied if the ATP was omitted from the experimental mixtures.

Similar results were obtained with soluble enzyme preparations from intestinal mucosa of rats and dogs. These preparations were made by scraping off the mucosa and freezing immediately with liquid air and pulverizing in a mortar. The powder while frozen was treated several times with cold (below −10° C) acetone, thereafter with ether, and dried in air. This acetone dry powder could be kept at temperatures below −10° C for 10–14 days without loss of activity. Soluble preparations were made by stirring the dry powder with ice-cold distilled water and centrifuging. The clear supernatant loses activity if stored more than 48 hr, even at temperatures below −10° C.

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Manuscript received March 27, 1953.

Comments and Communications

The Tension Concept in Aquatic Biology

STUDENTS of animal respiration have long used the tension concept to express the pressure of O_2 or CO_2 in body fluids. (Tension = partial pressure of O_2 or CO_2 in the gas phase at equilibrium between gas and liquid phases.) For terrestrial animals the concept is realistic and valuable because an exchange of gases between gas and liquid phases is essential to the respiration process. But in aquatic animals, where movement of CO_2 and O_2 is confined to the liquid phase, the tension concept is superfluous and even misleading. It is superfluous because the O_2 and CO_2 contents of the environment can be measured directly, and misleading because it leaves the erroneous impression that exchange of dissolved gases between animal and environment is similar to the exchange between gas and liquid phases. When respiration in aquatic environments is studied at constant temperature, the relation between tension and concentration is a constant one, and no serious ambiguity results; but when temperature varies, the tension-concentration relationship, of course, varies greatly as a result of changing solubilities of O_2 and CO_2.

These facts are well known, but their importance has not always been evaluated carefully. An example of such failure appears on pages 242–243 of *Comparative Animal Physiology* (Prosser, Brown, Bishop, Jahn and Wullf [Saunders, 1950]) where, in Fig. 53, a graph of Fry and Hart (The relation of temperature to oxygen consumption in the goldfish, *Biol. Bull.*, **94**, 66–77 [1948]) is reproduced. The graph shows the relation between oxygen tension and consumption in a goldfish exposed to temperatures varying between 5° and 35° C. From this graph Prosser *et al.* draw the conclusion: "At reduced tensions the oxygen consumption rapidly falls off, being affected at a higher oxygen tension for the higher metabolic rate, indicating the inability of the fish to maintain the specific activity at the designated temperature." But if this graph is reconstructed using concentrations, i.e., oxygen in solution, instead of tensions, the trend practically disappears, and the critical oxygen concentration proves to be about 60 μmol per liter regardless of temperature (or the fish's activity). The relative constancy of this critical oxygen concentration indicates that diffusion across the gill membranes was not an important limiting factor in O_2-uptake during those experiments—a significant implication of the data which Prosser *et al.* have missed while discussing a trend which was primarily an artifact of changing solubility.

The text goes on to say that one should not ignore the changing solubility of oxygen, and the increase in activity, with temperature. But no evaluation of these factors is made. One is left with the impression that these variables cannot be isolated. The authors could profitably have turned back to pages 210–211, where they reproduce Fick's law, and where they discuss the influence of temperature on the "diffusion constant." Fick's law employs concentrations, not tensions, thus the influence of solubility is taken into consideration simply by determining the concentration. The influence of temperature on the diffusivity or "diffusion constant" can also be estimated (about 1% per degree near 20° C) so the product Concentration × Diffusivity provides an expression of oxygen

availability which identifies the quantities and forces that operate.

Further inspection of Fick's law reveals that the change in diffusivity with temperature is only partly (about 0.3% per degree near 20° C) explained by the proportionality with absolute temperature (T). The major influence must be associated with changes in frictional resistance (f) of the medium with temperature.

The above comments will serve to emphasize that the tension concept is not useful when one is concerned with diffusion within the liquid phase. Concentration units should be used in such situations, and if one wishes to account for changes in diffusivity the product Concentration × Diffusivity will provide the desired index.

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Received December 1, 1952.

Treatment of Mouse-Passaged Dengue Virus (Mochizuki-strain) with Protamine Sulfate

IT has been reported that certain viruses are precipitated by adding protamine sulfate to the crude suspensions (*1–3*), and that many species of virus fall into two groups, with few exceptions, i.e., those protamine-precipitated, of larger size, and those protamine-non-precipitated, of smaller size (*3*). Nakagawa (*4*) reported his results of the application of protamine to the Hawaiian strain dengue virus isolated by Sabin and Schlesinger (*5*). The author also, independently of Nakagawa, conducted experiments on the protamine treatment of the mouse-passaged Mochizuki-strain dengue virus (*6*).

From mice moribund after an intracerebral infection of dengue virus, the brains were removed and ground into a 20% emulsion with distilled water.[1] The emulsion was centrifuged at 3,000 rpm for 15 min, and the supernatant fluid taken out was the original virus suspension. Protamine sulfate (clupeine, powdered)[2] was added to the virus suspension at the ratio of 5 mg/ml. The mixture, containing flocculates formed, was set at 0° C for 24 hr, then centrifuged at 3,000 rpm for 60 min. The supernatant thus obtained was slightly opaque fluid. Virus concentrations of the original suspensions as well as the supernatant were measured by an ordinary ten-fold titration in mouse brain. Additionally, the nitrogen amounts of these materials were determined by the micro-Kjehldahl method.

TABLE 1

VIRAL CONCENTRATIONS AND NITROGEN AMOUNTS OF PROTAMINE-TREATED MATERIALS

Expt. No.	Crude suspensions		Protamine supernatants	
	LD50* (L_{50}g)	Nitrogen (mg/ml)	LD50* (Log)	Nitrogen† (mg/ml)
I	5.7	1.512	5.7	1.028
II	6.0	1.538	5.9	1.176
III	6.0	1.521	6.0	1.085

* By the Reed-Muench method (7).
† Containing the excess of protamine.

[1] Adjusted to be pH 7.0 with sodium bicarbonate.
[2] Prepared by the Kossel method at Kyoto University Department of Biochemistry.

Results obtained are summarized in Table 1, and indicate that (1) dengue virus remains abundantly in the protamine-supernatants, (2) therefore, it can be included in the Warren's "protamine-non-precipitated" group of viruses (*3*).

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[3] The author wishes to express his gratitude to Professor Ren Kimura, director of the Institute, for helpful advice in the experimentation. He is also indebted to Mr. Atsushi Sugii and Miss Miyo Sasaki for their technical assistance.

Received January 30, 1953.

Evidence for the Nonexistence of a New Highly Penetrating Component of the Solar Radiation

IN 1941, M. Takata and T. Murasugi (*Bioklim. Beiblätter der Meteorologischen Zeitschrift*, 8, 17–26, [1941]) had described a very surprising discovery. These Japanese authors had found that the amount of $HgCl_2$ solution which has to be added to produce protein flocculation in diluted human blood serum varies approximately 10 to 15% dependent upon whether the blood, from which the serum is prepared, is taken from the vein some minutes before or some minutes after the sunrise. It was particularly interesting that this effect (called by Takata the "cosmoterrestrical effect") is noted only if the person is electrically insulated from ground during the taking of blood. Furthermore, the effect is also present in the same intensity, if the person is in a deep cellar room protected from any solar radiation by thick layers of earth and concrete. So, it seemed that a very penetrating and, before Takata's work, absolutely unknown component of solar radiation was found for which only the biochemical test of Takata, but no known physical experiment, gave evidence. Because of the highly interesting physical aspect of the findings