chlororaphine,[2] form a semiquinone radical as an intermediate state of reduction, it was suggestive to search for such a reaction in the closely related group of dyes consisting of the derivatives of isoalloxazine, including what is called lactoflavin, the dye-stuff component of Warburg's yellow respiration enzyme,[3] and identified with vitamin B_2 by Kuhn.[4] In fact, Kuhn and Wagner-Jauregg[5] showed that in a very acid solution an intermediate red form of the dye can be observed. At pH >1 no trace of this red form could be found.

Now, in the case of pyocyanine it has been shown[6] by a potentiometric method that even around neutrality a definite although small amount of the semiquinone can exist in equilibrium with the oxidized and the reduced form. Accordingly, Kuhn and Moruzzi,[7] Stern[8] and later Stare[9] have attempted to show that the same holds for the flavines. They applied the potentiometric method, basing calculations on what has been called the index potential.[10] Stern especially made much use of this method. In a paper to be published soon it will be shown that qualitatively the results of these authors are acceptable, those obtained by Stern even more so than those obtained by Stare, but quantitatively they have to be subjected to considerable improvement before they finally lead to a clear picture of the situation. However, even this evidence is based on very delicate observations of the index potentials in that range of its value where an error of a few tenths of a millivolt makes a big difference in the equilibrium percentage of semiquinone. Under these circumstances it is desirable to have supplementary even though only qualitative evidence for the existence of the semiquinone in neutral solutions. The following experiment gives satisfactory evidence.

A solution of any representative of the flavines (including vitamin B_2), saturated at 70°–80° C., in a buffer anywhere between pH = 4 and 10, and always kept about this temperature to avoid precipitation, is mixed with a suitable amount of solid sodium hydrosulfite. The color changes from intensely yellow through a dirty olive green to pale yellow, and on reoxidation by air the whole color change is reversed.

The color of the semiquinone in approximately neutral solution is, therefore, green. In very acid solution it is red. This is due to a different state of ionization, there being a dissociation constant of the semiquinone, pK = 1.0, approximately. In the very low concentration obtainable at room temperature in weakly acid or neutral solution it is more difficult to observe this phenomenon, although it can be observed on looking through the whole length of a test-tube.

The existence of this intermediate form in neutral solution, which previously rested on rather tenuous evidence, has now been made much more certain by this color reaction. It is easy to imagine that this property, in general very rare in dye-stuffs and just encountered in a group of dyes, one of which has such an important physiological property, if it contains a side chain of a definite steric structure (d-Ribose), should be of physiological significance. It is suggestive to think that in some cases the active form of certain enzymes might be the semiquinoid form. Here, a definite level of *oxidation-reduction,* observed in only a few dyes, is the active form, in analogy to the fact that in some other enzymes one definite state of *ionization,* as determined by the pH, is the kinetically active form.

L. MICHAELIS
M. P. SCHUBERT
C. V. SMYTHE

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH

[2] B. Elema, *Rec. trav. Chim. Pay-Bas,* 52: 569, 1933.
[3] W. Warburg and W. Christian, *Biochem. Zeits.,* 266: 377, 1933.
[4] R. Kuhn, P. György and T. Wagner-Jauregg, *Ber.,* 66: 317, 1933.
[5] R. Kuhn and T. Wagner-Jauregg, *Ber.,* 67B: 361, 1934.
[6] L. Michaelis, E. S. Hill and M. P. Schubert, *Biochem. Zeitschr.,* 250: 564, 1932.
[7] R. Kuhn and G. Moruzzi, *Ber.,* 67B, 1220, 1934.
[8] K. G. Stern, *Biochem. Jour.,* 28: 949, 1934.
[9] F. J. Stare, *Jour. Biol. Chem.,* 112: 233, 1936.
[10] L. Michaelis, *Jour. Biol. Chem.,* 96: 703, 1932.

ELECTRICAL BRAIN WAVES AND TEMPERATURE

IN a previous note in this journal,[1] I reported that values of the critical thermal increment of approximately 8, 11 and 16 thousand calories had been found for the frequencies of the alpha rhythm (most commonly called the "Berger rhythm" by Adrian and others) in a group of 6 patients whose temperatures were elevated by diathermy. Recently Jasper[2] has criticized the identification of these values, since he finds fluctuations of several cycles per second in a run of an hour or two without, he says, any temperature change.

Fig. 1 is a plot according to the Arrhenius equation of frequencies (F) of the alpha cycles as a function of absolute temperatures (T). If the equation fits, one should get a straight line of negative slope by plotting log F against $1/T$. μ in calories is determined directly from the slope of the line. All the data involved in my first report are embodied in this figure as well as data from 4 additional subjects—10 in all. The lower curve of the figure ($\mu = 8000$ calories) corresponds to 7 daily experiments on 3 normals, 8 daily experiments on 2 mild general paretics and 5

[1] H. Hoagland, SCIENCE, 83: 84–85, 1936.
[2] H. H. Jasper, SCIENCE, 83: 259–260, 1936.

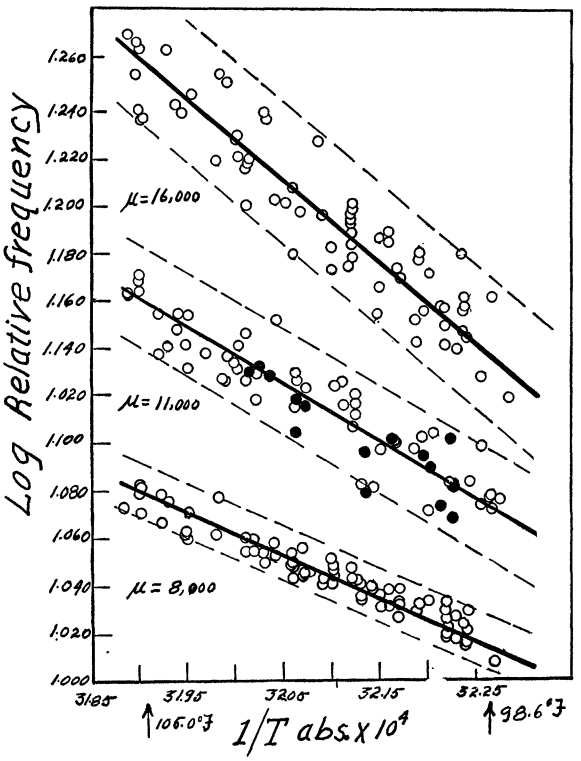

FIG. 1.

experiments (each a week apart) on a multiple sclerosis out-patient. Each experiment involves from 5 to 9 points; approximately 500 alpha waves are averaged for each point in Fig. 1.

The relative *variability* is shown by the vertical height of the band between the two parallel broken lines on either side of the heavy mean line. The $\mu = 11{,}000$ band corresponds likewise to 11 experiments on 2 general paretics who have had the disease longer than those of the 8,000 group. The $\mu = 16{,}000$ band corresponds to 14 experiments on the 2 other general paretics. One of these was decidedly the most advanced case of the group. The clinical record of the other, while showing an advanced stage, was incomplete and did not permit one to say that the paresis was more advanced than in the 11,000 group. The clinical records were independently analyzed by Dr. F. H. Sleeper. Of course, many more cases are necessary before clinical generalizations can be made. Some 115,000 alpha rhythms have gone into the determinations. Individual experiments are brought together in the band of appropriate slope by multiplying ordinates by a suitable constant. The ordinate intercept of each group is, therefore, arbitrary. The slopes alone are here significant. The 15 solid black points of the $\mu = 11{,}000$ curve show frequencies as a function of *descending* temperatures in 4 experiments. No hysteresis effect is seen, thus indicating the quantitative *specificity* of the temperature effect and showing that any small downward drift in frequency which may occur in time at normal body temperature is quantitatively overcome when the underlying mechanisms are driven by elevated temperatures. The slight decline in frequency after 1 to 2 hours recorded by Loomis, Harvey and Hobart[3] may possibly be due to a corresponding fall in temperature as basal metabolic conditions are approached.

There is no question of the independence of the three μ values as evidenced by the three distinct slopes of the lines. It is interesting that the variability represented by the width of the parallel bands apparently increases with the advancement of general paresis. The banded form of the variability means that the relative (per cent.) variability is constant over the temperature range for each graph. The variability is, of course, organic and far exceeds errors of measurements and is of the type commonly found in studies of physiological rates as a function of temperature. The specific factors making for excessive variability listed by Jasper were avoided to a great extent in the series. The *reversible* fit of the Arrhenius equation, the specific μ's which correspond to numerous determinations for O_2 consumption and CO_2 production in cells and the measured variability thus obviate the criticisms. In addition, Jasper's findings do in fact support our own. His values of 7,000–8,000 calories for normals and petit mals agree well with ours for the normals, the multiple sclerosis patient and the two least affected general paretics. The higher values of 11,000 and 16,000 appear to be products of the advancement of the infection which might quite reasonably be expected to shift the pacemaker, *i.e.*, the slowest process in the sequence of cortical cell respiratory events determining the relaxation oscillation frequencies. Work with poikilothermous organisms has repeatedly shown similar shifts due to chemical manipulation from one of these three values to another (Crozier and Stier).[4] Jasper agrees with me in favoring the idea of a relaxation oscillator mechanism for the brain waves, but for different reasons. It would indeed be surprising, granting such a mechanism, if the Arrhenius equation did not fit the data.

HUDSON HOAGLAND
CLARK UNIVERSITY

PYRUVIC ACID IN URINE AFTER HARD EXERCISE

DURING nine experiments in which athletic young men ran quickly to exhaustion on a treadmill, we col-

[3] A. L. Loomis, E. N. Harvey, G. Hobart, SCIENCE, 83: 239–241, 1936.

[4] W. J. Crozier and T. J. B. Stier, *Jour. Gen. Physiol.*, 9: 547–559, 1926.