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SOME NOTES ON THE PROBLEM OF UNCERTAINTY IN BIOLOGICAL MEASUREMENTS

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An Introductory Note: *Some time ago, when Dr. Harold Morowitz was engaged in bio-instrumentation work at the National Heart Institute, he wrote two articles encompassing the subject of these notes: one, "Measurement in Biology" appeared in the Proceedings of the 1953 ISA annual meeting, the other, "The Relationship Between the Process of Measurement and Instrumentation in Biology" was printed as article 6 in Volume 60 of the Annals of the New York Academy of Science. When BIAC was established over ten years later these papers seemed to have more relevance to the problems we face in bio-instrumentation than ever. So we put together a special synthesis of the two as a general review and sermon for both biologists and their engineer-collaborators alike.*

The success of precise quantitative measurement in the physical sciences has prompted many biological research workers to attempt quantitative measurements in their respective disciplines. A growing number of investigators are engaged in an attempt to analyze complex biological phenomena in terms of the simpler laws of physics and chemistry. This trend toward physical theory has been accompanied by an ever-increasing use, by biologists, of instruments and measuring techniques originally developed for other purposes.

However, in spite of the extensive application of modern instrument techniques, biology is far from its goal of being a quantitative science. An instrument is of value only in so far as we are able to understand or use the resultant measurement. Biology presents special problems in evaluation of such measurements. A large number of measurements obtainable to several significant figures are meaningless in terms of interpretation and reproducibility. Much of this uncertainty arises from confusion regarding the fundamental nature of a measurement on a biological system.

Two related questions arise in considering the problem of biological measurement:

1. What are the criteria and meaning of an individual measurement?

2. What type of analysis of data is suitable to the limitations which arise in the consideration of the preceding question?

It is the purpose of this paper to examine the first question in detail in an effort to study the limitations and possibilities inherent in instrumentation advances. In addition to examining the measurement process, this paper presents a brief treatment of a rudimentary case; where information theory points the way to qualitatively evaluating the limitations of biological measurement.

ASSUMPTIONS

In the following, it is assumed that biological systems follow the normal laws of physics and chemistry. The difference between biological systems and ordinary physical systems is assumed to be the result of the tremendous complexity of the former.

In dealing with measurements we will employ an operational concept closely akin to that of Bridgman. A measurement is a specified set of operations which gives rise to a coincidence to which we assign a numerical value. The instrument will also be considered as an operationally defined entity.

THE MEASUREMENT COMPLEX

In performing a measurement on experimental material, little significance can be attached to the measurement of a single parameter. To take a specific example, consider the measurement of viscosity. In order to attach significance to the value obtained, it is necessary to specify temperature and possibly rate of shear. However, the additional quantities specified are themselves the result of measurements which ideally should be carried out simultaneously with the desired measurement. Thus, the ultimate item to be obtained from the measuring process is, in general, not a single measurement but a measurement complex consisting of the simultaneous measurement of all independent variables.

In the formulation of thermodynamics, two independent variables are sufficient to specify the state of a one component system. In such systems, the necessity of considering all independent variables seldom gives rise to difficulties. Even in more complicated cases dealt with in physics or chemistry, the number of independent variables is seldom more than six or eight. As a consequence of this relative simplicity, a very fruitful methodological approach has been developed. It is based on the idea of limiting the number of variables under consideration. The ideal experiment in this conceptual scheme consists of measuring the variation of one measurable property as a function of the variation of a second measurable property, while keeping all other possible independent variables constant. The resultant relationship between the two variables, which constitutes the raw material for scientific theories, can be expressed in the thermodynamic parlance in the following form:

$$\frac{\partial X_1}{\partial X_2} \quad x_3 \quad x_4 \quad \ldots\ldots \quad x_m = f(X_2)$$

The assumption is made that the system is in equilibrium or in a steady state while the measurement is being made.

In most biological measurements, the number of apparently independent variables becomes very large; and the relative simplicity disappears. In addition, the situation is often made worse by the fact that many of the independent variables are unknown or ill understood.

As an example of the large number of apparently independent variables which may enter into a biological experiment, let us consider the determination of the blood pressure of a mouse as a function of external temperature. Assuming an adequate pressure measuring instrument were available, the following are some of the apparently independent variables:

1. Humidity
2. Composition of inspired air
3. Weight of mouse
4. Last feeding time of mouse and composition of food
5. Age of mouse
6. Activity of mouse during experiment
7. Time (since the system is not in equilibrium, time enters as an independent variable.)
8. Thickness of coat of hair on the mouse
9. Sex of mouse

Each reader, according to his own experience, can add many more variables to this list. In addition, in all probability, the experiment would not be reproducible. If the external temperature was gradually increased from 0° to 50°C and the experiment was repeated at some subsequent time, different relationships between pressure and temperature would most probably be found in the two cases.

Thus, one difference between physical and biological measurement is in the small number of well controlled variables in the former and large number of poorly controlled variables in the latter. Since a measurement in the physical sense implies control or measurement of all variables in the complex, it may be seen that the meaning of measurement is altered in biology.

UNCERTAINTY

The process of measurement always produces, to a greater or lesser degree, changes in the material being measured. If these changes are large, the system undergoes rapid transformation and there is an uncertainty as to whether the measurement taken applies to the system as it existed at the beginning of the measurement. The uncertainty in physics has become a fundamental part of the description of atomic processes. Biology, thus far, offers no quantitative method of dealing with the particular type of uncertainty which arises in measurements on living systems.

There are two chief aspects concerning biological uncertainty, one on the micro level and the second on both the micro and macro levels. At the micro level, we have the problem of a complete functioning cell being the same order of size as the smallest probe we can make for a measuring instrument. All measurements are thus either indirect or accompanied by a high degree of uncertainty.

In addition, for all levels of size, the measurement process may act as a stimulus causing very great changes in the object measured.

The general character of a stimulus response situation is such that a very small amount of input energy causes large changes in the organism. Reducing the input energy coming from the measurement process does not help greatly, unless it is reduced below the threshold level for stimulation. The energy changes of a stimulus response situation are usually only possible in a system at a high potential.

A measurement of an inorganic solution showing stimulus response characteristics would perhaps elucidate the previous discussion. Consider the measurement of the electrical conductivity of a super saturated salt solution. Placing the conductivity meter probe into the solution causes salt to suddenly precipitate, changing the system and causing a resultant uncertainty in the measurement of the conductivity of the super saturated solution.

Situations similar to the above mentioned case occur frequently in biological measurements. Irritability is a fundamental feature of biological systems and must be faced in all measurements.

THE PROBLEM

Measurement in the classical physical sense is thus limited in biology in the following rather parodoxical fashion. A single measurement complex requires a large number of simultaneous measurements, each of which produces an uncertainty in the results. The effect of the uncertainties may be cumulative and lead to a large uncertainty in the final result. These difficulties are inherent in the complexity and instability (from a thermodynamic point of view) of biological systems, and refinement of measurement can never completely overcome the trouble.

One mode of approach is to assume that the epistemological relationship between theory and experiment is different in biology and physics. This leads to the necessity of formulating biological theory in terms of the new point of view. Since epistemological correlation is one of the most uncertain points in the philosophy of science, grave difficulties are to be anticipated in this approach.

Another approach is somewhat indirect and deductive in nature. It is possible to measure, in the usual physical sense, the material and energy input and output of a biological system under varying external conditions without taking direct measurements on the living biological material. The job of theory is then to postulate a biological system consistent with these measurements. This is much the same point of view as has been adopted in postulating the structure of molecules from data on radiation absorbed and emitted. Such a methodology is quite different from the usual biological point of view.

A third approach is to devise methods of handling data which are consistent with the limitations and uncertainties inherent in individual measurements. Such an approach is contained in many of the statistical methods developed in recent years. Careful consideration must be given to the type of analysis most likely to be successful in dealing with the results of biological measurement.

AN APPROACH TO THE UNCERTAINTY PROBLEM

Returning to the problem of determing the uncertainty accompanying biological measurement, informative theory suggests the possibility of a quantitative approach. Consider for the simplest case, a single cell of information content I. The atomic constituents of the cell can exist in n possible states, of which L states correspond to the cell being alive and normally functioning. We may then define the information content of the living cell so that

$$I = -\log_2 \frac{L}{n}$$

Information and negentropy of formation are so related that the latter is given by the following expression

$$N = .6931\ kI$$

where k is Boltzmann's constant and $.6931$ is the natural logarithm of 2. This analysis is based on the first order of approximation postulate that all atomic states of the system have the same priori probability. Accompanying a given measurement, there will usually be an increase in entropy ΔS, such that the final negentropy of formation will be

$$\begin{aligned} N' &= N - \Delta S \\ &= -.6931k \ \log_2 \frac{L}{n} - .6931k \ \log_2 \alpha \\ &= -.6931k \ \log_2 \frac{L\alpha}{n} \end{aligned}$$

where $\Delta S = k \log \alpha$.

Prior to the measurement, the system was in one of L states all corresponding to normal living systems. After the measurement, it was in one of $L\alpha$ states. The probability of the system remaining in one of the original states is them

$$p = \frac{L}{L\alpha} = \frac{1}{\alpha} = e^{\frac{-\Delta S}{k}}$$

p is the probability of the cell being unchanged by the measurement. The larger the value of ΔS, the smaller the value of p, and the greater the uncertainty of the measurement.

While such analysis omits many of the details accompanying biological measurements, it provides a heuristic approach to the formulation of a quantitative uncertainty principle for biology. One of the assumptions causing a good deal of uncertainty is the one regarding the same priori probability of all states. This assumption is made to simplify the presentation. Any actual case to be considered would involve considerable detail in setting up an expression for the information content.

BESIDES UNCERTAINTY

The chief factor which distinguishes biological instrumentation from conventional types of instrumentation is the design of the sensing probe. The other components of the instrument system, such as communication channel, amplifier, recorder, and observer are common problems shared by various fields of instrumentation. In addition, it should be realized that, for many problems of biological measurement, the instrumentation is adequate, but the preparation of the material requires critical insight if the measurement is to have significance.

The design of the probe requires certain general considerations:

(1) *Minimum perturbation.* Often a compromise must be made between sensitivity and precision on the one hand and probe size on the other.

(2) *Precision and variance.* The variance of the measured quantity should determine the necessary precision. It is uneconomical to strive for greater precision in a single measurement than is justified by the variance of a set of measurements.

(3) *Signal sensitivity.* After a decision has been made as to what physical quantity the probe is to measure, it must be designed so that variations in other physical quantities have a minimum effect on the output. This is also true in nonbiological systems.

Approaching the subject from a more practical level, it would seem that the problem of biological instrumentation is a part of the larger problem of developing a discipline to give meaning to biological measurement. The responsibility of deciding which measurements to make (which sensing elements to use and how to make them interact with the systems to be measured) is therefore the biologist's problem. For it is only within the context of the theory of a given discipline that a particular set of operations can yield interpretable data. Measuring techniques must be evolved to fit each problem, and the biologist should not expect to buy his research instruments ready made any more than a physicist should so expect. The great advances in experimental physics have not come from men who have purchased a commercial instrument and taken measurements. They have rather come from men like Millikan, with his oil drop apparatus, or Michelson and Morley, with their optical equipment. The advances have come when men have designed a specific apparatus to provide an answer to a question framed by a background of theory. It would also seem that biologists must design apparatus consistent with a theoretical framework and that the subsequent measurements are interpretable or at least meaningful. To expect that unmodified commercial instruments will always serve in this way is a surrender of the biologist's claim to understanding his own field or, at least, an admission that the instrument manufacturer has a better understanding of biological problems than that possessed by the instrument user.

This assertion is not to deny that a great deal of very valuable research is performed with commercial equipment. It is rather to assert that a measurement is not an act in isolation, but must be connected with a discipline which gives meaning to the measurement. This connection is clearly the responsibility of those trying to develop the discipline and take the measurements.

What function does this leave the instrument engineer who wishes to contribute to biological instrumentation? I think that there are three possibilities for such an individual: First, he may become a biologist in the sense that he may learn enough biology to develop instruments and techniques which make sense for their biological applications, Second, he may act in a consulting capacity in advising the biologist what is available in terms of transducers, amplifiers, and the like. Third, he may devote himself to that part of the system which is common to all instrument systems and may aid in that part of a project.

The biologist, on the other hand, must acquaint himself sufficiently with optics, mechanics, electronics, and so forth, to be able to understand the design of his experiment. Here, I think that the education of biologists is subject to improvement. The inclusion of a university course for biologists on basic instrumentation, instrument design and use, and the theory of measurement should aid the biologist in developing new techniques to answer new questions.

A FINAL WORD

The preceding analysis may appear discouraging to those who look to instrumentation to solve the many baffling mysteries of living systems. Rather than serving as a discouragement, an understanding of the difficulties should serve as a challenge to those who would attempt to understand biological phenomena in terms of quantitative data.

The problems of biological instrumentation go to the core of our understanding of biological processes. Judging which measurements are significant implies an understanding of the significant questions which a science may pose, and this understanding implies considerable maturity and theoretical foundation. Biological instrumentation cannot be separated from biological theory, for the meaning of each measurement must be sought in the instrument, the measuring process, and the nature of the material being measured.

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