A Background for Biological Studies with Radioiodine

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PEOPLE WHO MAKE HISTORY, it is said, do not have the time to read it. Nevertheless, a little perspective is a good thing in assessing the value of one's work and in indicating which experiments it is important to pursue. This is particularly true of thyroid physiology because of the rapid speed with which studies involving radioactive iodine have progressed. For instance, my monograph published in 1940 (*21*) includes this statement:

> *Radiation Therapy with Radioactive Iodine.* It has been suggested by a number of investigators that in thyroid hyperplasia and neoplasia it might be possible to treat the disease with iodine, which, being radioactive, would be trapped in the thyroid tissue and would disintegrate there close to the site of the proliferating cells. . . .

It is interesting that this pipe dream, which seemed on the edge of the fantastic at the turn of the present decade, is now rapidly coming true.

Correlation with the past. Anyone familiar with investigations in iodine over a period of years becomes impressed by the fact that often the left hand doth not know what the right hand doeth. An illustration is the discovery of iodine, nearly one hundred and forty years ago. During the Napoleonic Wars the emperor was short of nitre with which to make gun powder, and his government subsidized a lot of little people known as *salpetriers* whose business it was to produce saltpeter. This they made from potash and later from kelp or a seaweed which they called *le varech* in the French vernacular. In 1811, a little manufacturer named Courtois discovered (*6*) that there was something in the liquors derived from his seaweed ash which corroded the copper lining of the vats. Out of this industrial problem came the isolation of a purple substance which the eminent French chemist Gay-Lussac (*8*) called iodine, from the Greek word ἰώδης which means *violet-like.*

By 1820 the Swiss physician Coindet (*5*) had published his classic paper on *"Découverte d'un nouveau remède contre le goître."* This remedy soon became so popular that in the French Midi legislation was passed prohibiting the indiscriminate use of the material. Everybody knew that iodine, which came from seaweed, was a remedy against goiter. Nevertheless, when Baumann (*1*) in 1895 published his classic paper *"Über das Normale Vorkommen von Jod im Thierkörper,"* he said, *"Als ich diese Beobachtung zuerst machte, glaubte ich an alles Andere eher, als dass Jod meiner Substanz angehöre"* (When first I made this observation, nothing was further from my mind, than that iodine was connected with my material).

Physical biology vs. biological physics. Speaking with nuclear physicists, biophysicists, clinicians, and various people associated through a common interest in radioiodine, one cannot help being impressed by the fact that a comprehensive view of the problem often would greatly assist their work. The physicist, for example, tends to imagine a human body built up of relays of electric bulbs, wire springs, and steel supports. He evolves theories about this imaginary robot which do not really fit the circumstances of flesh and blood. The clinician, on the other hand, is often just as remiss in his naive way of applying physical tools to a tracer problem. One of the important consequences of the symposia that are frequently held today on biological radioactivity is the opportunity they present for a meeting of minds reared under variegated disciplines.

Questions to be asked and answered. An important question that I hope will be brought out in modern investigations is the question of the sweet reasonableness of the results. In other words, how do the data newly presented harmonize with the old facts established by classical chemical methods? In a recent review (*24*) I have attempted to interpret in physiologic terms some of the data on radioactivity now in the literature. Unfortunately, a good many findings are entirely empiric and have no obvious physiologic meaning. In part this is due to the fact that the data on radioactivity have not been integrated with a supportive program of biochemical study. Furthermore, in part the data derived by isotope studies have been confused by technical problems.

This betrayal of the scientist by his armamentarium is no new problem. About 1850, for example, the French scientist Chatin developed a micromethod for measuring iodine. He applied it in a comprehensive study of the waters of the various valleys of the Rhone, the Seine, and other rivers of France. Out of this work he drew the surprisingly clear statement

(*4*) that the principal cause of goiter and cretinism was an insufficiency of iodine. Moreover, he went on to say that it would be a simple matter to reinforce the deficient sources of water supply with mineralized solutions of iodide (*3*). His fellow scientists tried to apply his method and failed. Finally, the French Academy surveyed these results and concluded that Chatin's work was not tenable. The poor man ended his career in disappointment and frustration; and the world remained indifferent to this important etiological factor in goiter until in rather recent decades the work of Marine (*14*), McClendon (*15*) and others eminently confirmed Chatin's hypothesis.

Similarly one finds today that, because of the inadequacy of biochemical and radioactive techniques, our modern scientists frequently disavow conclusions which are plainly true in the light of data established by the older classical methods.

The integration of older observations with modern findings. Chatin's contemporaries laid too much emphasis upon negative technical findings. In so doing they ignored a train of independent observations covering a span of 17 centuries. I can mention only a few of these. In the first century A.D., Pliny the Elder (*18*) noted that goiter occurred in certain localities and stated that the waters of these resembled the land. *"Tales sunt aquae quales terrae, per quas fluunt."* In medieval times sponge had been used to treat goiter (*26*), and later had been incorporated in the "Coventry" treatment used in 18th century England (*27*). In Renaissance times Michelangelo (*16*) complained to his friend Giovanni da Pistoja that he had grown a goiter while painting in the Sistine Chapel—just as a cat in Lombardy might:

> I'ho gìa fatto un gozzo in questo stento,
> come fa l'acqua a' gatti in Lombardia,
> ovver d'altro paese che si sia,
> ch' a forza il ventre appicca sotto il mento.
>
> I've grown a goiter by dwelling in this den—
> As cats from stagnant streams in Lombardy,
> Or in what other land they hap to be—
> Which drives the belly close beneath the chin.

In 1812 Courtois (*6*) had discovered iodine in seaweed, and in 1809 the London Medical Dictionary (*17*) had recommended sea water to treat goiter. One wonders, now, how the leading French scientists dared to discredit Chatin's conclusions. Of course, hindsight is ever more lucid that foresight and it is all too easy to scoff in retrospect. As he picks up his beautiful new tool, however, it is well for the modern biologist to remind himself how subtly and completely a fascination for gadgets can betray sound sense.

Evaluation of tools and methods. Part of the difficulty lies in a misplaced confidence in one's technique, or perhaps a failure to appraise the limitations of methodology. Again, history supplies an illustration. In 1848, King (*10*) at Guy's Hospital in London had hypothecated the existence of an internal secretion derived from the thyroid:

> . . . we may one day be able to shew, that a particular material principle is slowly formed and partially kept in reserve; and that this principle is also supplementary when poured into the descending cava, to important subsequent functions in the course of circulation.

Moreover about 1883, the rival Swiss surgeons Kocher of Berne and Reverdin of Geneva had agreed (*11, 20*) that the more nearly complete their thyroidectomy, the worse for the patient ultimately. After Sir William Gull (*9*) had described the widespread tissue changes in human myxedema and Fox (*7*) and Mackenzie (*13*) had demonstrated the reversal of these changes by the feeding of thyroid, Baumann (*1*) showed that the essential substance "thyroidin" contained iodine. It should have been obvious that an iodine-containing hormone normally circulates in the blood stream. Nevertheless, as late as 1914 it was believed (*2*) that the normal plasma contained no iodine in organic combination. The discrepancy was resolved only after improved techniques had been devised. Similarly, in the present decade the earlier publications on radioactive iodine frequently constituted pharmacologic rather than physiologic endeavors; because too much carrier (inert) iodide accompanied the so-called "tracer." Therefore, we must endeavor to bring out the answer to the question: How do these data harmonize with the established knowledge of thyroid physiology and iodine metabolism already developed painstakingly by classical methods of biochemistry?

Newer knowledge. We must also ask what new concepts have evolved from the use of radioactive iodine that were not established earlier. Obviously this new tool—perhaps the most important scientific weapon since the discovery of the microscope—provides the opportunity for a kaleidoscopic or cinematographic picture of metabolic events. By making repeated observations in a single animal or man, one can obtain a picture which formerly would have required the sacrifice of whole series of test animals. Nevertheless, it is a little disappointing to find some modern isotope-minded physiologists content with developing a series of data and proclaiming conclusions which are already well established in the literature of thirty years ago. Granted that the older data were built of painstaking analyses on single animals and the composite picture which had to be drawn therefrom; yet the final concepts—the heart of the problem—often have already been resolved by older techniques.

This problem of rediscovering what was long since established is not new. In October 1863 the eminent French clinician Trousseau (*25*) inadvertently gave a patient with exophthalmic goiter some tincture of iodine when he meant to write tincture of digitalis on the prescription blank. He did this because he knew subconsciously that iodine was traditionally a bad drug for these people. When the patient returned to his office, however, she was much improved. Discovering his mistake, the physician changed to tincture of digitalis and the patient rapidly became worse. In short, before the time of the American War between the States it had been demonstrated clearly that iodine was a good remedy in hyperthyroidism. Nevertheless, when Henry Plummer (*19*) in the third decade of this century emphasized the use of iodine in such cases, the world hailed this as a novel approach. Circumstances change but the general pattern is the same. Men do over and over again what their predecessors did two generations ahead of them because the train of history is lost.

One of the most important things which the isotope technique can give us, besides localization of the material anatomically, is the indication of its rate of metabolic turnover. Such measurements, however, cannot be established through isotopes alone. In order to establish turnover rates it is usually necessary to have simultaneous measurements of both stable isotope and tracer. It is surprising how many physiologists, biochemists, and clinical investigators still rely solely on determining radioactivity, without supportive biochemical analyses of the classical type.

Combined chemical and physical approaches. There is a tendency in present-day tracer studies to rely on biophysical measurements alone, without accompanying chemical identification (either qualitative or quantitative) of the substance under observation. For instance, the thyroid gland normally contains iodine in three chemical forms; but rarely are they separated before measurements of radioactivity are made (*12*). Moreover, the fact is frequently overlooked that in order to interpret measurements of radioactivity, simultaneous measurements must be made of the inert iodine present. In other words, it is frequently necessary to know the *specific radioactivity* of the chemical substance or fraction under study. Consider the blood plasma of the normal rat, with a steady concentration of "hormonal" iodine H_o at about 2.5 micrograms percent. If one is to estimate the turnover rate of this hormone by administering radioiodide, one must combine data on radioactivity with microchemical analyses because the hormonal concentration represents a dynamic equilibrium (*22*). As fast as a little new radioactive hormone is formed, part of it is metabolized together with the previously extant hormone. This relationship is represented by the equation

$$\frac{dH}{dt}\left(1-\frac{L}{H_o}\right)=\frac{dL}{dt},$$

where H is the newly formed hormone and L the labeled hormone unexpended. At any given moment L can be evaluated from measurements of A, the radioactivity observed, and from σ, the specific radioactivity of the body's reserve of iodide. As a first approximation, these can be described by the following equations:

$$\frac{-d\, log\, \sigma}{dt}=k,$$

$$\frac{dL}{dt}=\frac{dA}{\sigma dt}=\frac{1}{K_o}\cdot e^{k_1 t}\frac{dA}{dt}.$$

Once L has been evaluated, the amount of newly made hormone can be calculated easily because

$$H_{t_o}=-2.5\, ln\left(1-\frac{L_{t_o}}{2.5}\right).$$

The ultimate solution for turnover rate, therefore, involves some sort of multiple integration. Without this mathematical approach much of the work which has appeared in the last few years cannot be interpreted physiologically (*23*). In general, for work with biological systems one cannot be content with isolated physical measurements presented on an empirical basis.

At this juncture, therefore, it is well to stop and think in terms of our accumulated knowledge of iodine metabolism and of thyroid physiology. Let us ferret out those new developments and new concepts for which the use of radioiodine is directly responsible. Let us inquire carefully into technical problems and means of solving them; but let us not be so diverted with gadgets that we forget the purposes which these tools might serve. Let us remember that biophysical methods must be adapted to the organism rather than the organism warped to fit oversimplified physical theory. If we bear these points in mind, we shall be able to bring out the answers to our chief questions, namely: 1) How has radioiodine improved our knowledge of iodine metabolism and of the physiology and the therapy of the thyroid? and 2) How do the results harmonize with past experience? If we understand clearly the answers to these questions it will be obvious what work must be done to make progress along modern biological lines.

Adapted from a paper presented at the Symposium on Radioiodine held at Brookhaven National Laboratory, Upton, New York during July 1948.

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Standardization of Radioactive Iodine

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THE TECHNIQUES AND INSTRUMENTATION of standardization that are to be described here are those available generally in laboratories equipped for work with radioactive materials in biology and medicine. Problems that can be solved by specialized and more complicated methods in a purely physical laboratory will be discussed only in a general way; however, such measurements as can and should be performed by every user of radioactive materials, especially I–131, will be presented in more detail.

The standardization of a physical object involves first of all a description of the measurement procedure and a definition of the unit to be used. The two are, however, not necessarily independent of each other; and we usually are free to select more than one procedure and unit.

Let us consider a piece of steel. We can standardize it by weighing, and the unit which we will assign to it by this procedure may be the kilogram. As is true of every good physical unit, there are several well-known measurement procedures for comparing our piece of steel to the physically permanent standard kilogram. The "weight" is an adequate standardization result if we want to use the piece of steel as ballast in a ship. Should we want it for casting, however, weight will not be the information required; we shall need its volume, expressed, let us say, in ml. To measure this directly is rather more complicated than weighing; we should not be permitted to use density, since "density" implies that a volume measurement and weighing have been performed on the identical steel at least once previously. If we plan to use the piece of steel as an armor plate, we shall need a different standardization, its thickness in cm, which can be easily measured by a micrometer. The situation will become more involved if we are to use the steel as a gamma-ray absorber. The micrometer, which gives the thickness in cm, will be very useful as long as we use the same steel alloy. But if we use different steel alloys, or even different materials, we find that the simple absorption equation is complicated by a coefficient that is characteristic for every material and varies widely. We know from our experience that the variation of this coefficient is reduced by at least one order of magnitude if we standardize the thickness of absorbing materials in g/cm^2 instead of cm. Such standardization involves more complicated measurements than the simple use of a micrometer. Yet this is what we do, for reasons familiar to us all.

The purpose of this example is to recall the multitude of possible standardizations on the same physical object and to show 1) how availability and simplicity of measurement procedures may determine the selection of units, and 2) that, depending on the ultimate use and application of the information supplied by standardization, one system of units may be exchanged for another, for simplicity and convenience.

In working with short-lived radioactive isotopes, a gravimetric unit for standardization is practically im-