Calorimetry and Metal Binding in Biology*

JAMES J. CHRISTENSEN**

President Wilkinson, honored guests, fellow faculty members, ladies and gentlemen: Dr. Izatt and I first wish to express our appreciation of the honor given us in being invited to present the Seventh Annual Faculty Lecture. It may appear strange to many of you that both of us were chosen to deliver this lecture. However, those of you who are familiar with our work know that we have collaborated for over 13 years in our research effort and that we are both involved in all areas of our research. We also find, in reviewing our work, that it is extremely difficult, if not impossible, to say that a given idea was due to one or the other of us. So we can see that the Annual Faculty Lecture Committee must have also had some difficulties, and although I have not verified this theory with the committee, I would surmise that they probably just threw up their hands when they came to our names and said, "Let's kill two birds with one stone." So here we are.

We would also like to acknowledge that most of our research would have been impossible to do if it were not for the many fine undergraduate, graduate and post doctoral students that have worked with us in the past years. They are also being honored here tonight.

We have divided the lecture into two parts: Calorimetry, about which I will talk, and Metal Binding in Biology which

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Dr. Izatt will discuss. In both our talks we have three central themes that we will be emphasizing. These are: our research activities, the scientific method of investigation, and the importance of metals in biology and specifically the human body.

I would like to tell you four short stories. See if you can determine what they have in common.

The first story concerns a farmer and his fruit orchard. In this western peach orchard the fruit trees were stunted and produced only a sparse crop of scrawny fruit until one year the farmer installed a galvanized fence around the orchard. That year, and in succeeding years, the yield of fruit was large and the fruit was of top quality.

The second story concerns a group of young track athletes from a high school in Los Angeles that won most of their home games, but *always* lost when bussed to a distant school for a track meet.

The third story concerns an experiment in which two groups of rats lived in wooden cages. One group breathed purified air and ate organic-grown, sterilized food, while the other group breathed normal air and ate normal food. After several months, 90 percent of the rats breathing the purified air and eating the specially prepared food were alive while only 43 percent of their brother rats in the other group were alive.

The fourth story concerns four companies of the second dragoons who arrived at Fort Randall on the banks of the Missouri River in Nebraska in 1856. About ten days later, most of the horses in the company experienced running at the nose, distemper, and loss of hoofs, manes and tails, followed by death after weeks or months. A similar experience was reported by Marco Polo in 1295. He noted that in the mountains of western China the most excellent kind of rhubarb was produced, but that merchants coming to bring it out could not allow their beasts of burden to graze in the surrounding mountains because the beasts would lose their hoofs.

The common thread running through these four stories is that each incident is related to the effects of metals on plants, animals or people.

In the first story, the ground was deficient in zinc causing the trees to have fewer and poorer fruit than normal. The galvanized fence was made of steel coated with zinc and

through the action of rain and of leaching by the soil enough zinc was transferred from the fence to the ground to meet the needs of the trees and to restore normal production.

In the second story, every time the track athletes were bussed along the busy freeways in Los Angeles, they breathed in larger than normal amounts of carbon monoxide. This carbon monoxide reacted with the iron in the hemoglobin in their blood to form carboxyhemoglobin. Every hemoglobin molecule that reacted with the carbon monoxide meant one less hemoglobin available to carry oxygen in the body. Consequently, the athletes were hampered by the reduced amount of oxygen supplied to the body and performed below their capabilities.

In the third story it is believed the lower death rate of the group of rats breathing purified air and eating specially prepared food was due to the absence of lead in the filtered air and food. From tree stumps it has been calculated that the lead concentration in the air today is 24 times the concentration of 100 years ago and a hundred times more than 1,000 years ago. This is not at all unbelievable if one realizes that 700 million pounds of lead is consumed every year in gasoline sales. An interesting theory has been proposed to explain the decline and fall of the Roman Empire based on the premise that the nobles and leaders were victims of lead poisoning. It was the custom of the time for the more well-to-do to have their wine sweetened by heating and storing it in lead pots. Lead was also used for cooking ware and for drinking cups. It is proposed that under these conditions enough lead was consumed to cause increased sterility in both men and women and to cause a larger than normal proportion of the children to be born with physical and mental defects. The leading class was therefore slowly reduced in numbers and leadership capabilities. Analysis of corpses from that time show an abnormally high lead content. (*Reader's Digest*, 1966; *Today's Health*, March 1966 and *C. & E. News*, March 9, 1970, p. 42.)

In the fourth story the animals were all suffering from selenium poisoning. The metal selenium occurs in high concentration in some soils and can be further concentrated by grains or grass. Even in fairly low concentrations, plants such as Woody Aster, Gray's Vetch and Astragalus concentrate selenium to several thousand parts per million part plant or up to 1 percent of the plant's weight can be selenium. In 1907-

1908 in a region north of Medicine Bow, Wyoming, 15,000 sheep died from selenium poisoning.

From these four stories it is possible to gain a glimpse of how important metals can be in the operation of living systems. Now not all metals are harmful, in fact, many metals are necessary for the correct operation of biological systems. Iron is just one example in the human body.

To understand the effects metals cause, it is necessary to know how metals interact with various systems. This is essentially what we are doing in our research in that we deal with the interactions in solution of metals with other molecules. The metals can be such things as Fe^{2+}, Na^+, Ca^{2+}, K^+, Zn^{2+}, Cu^{2+}, Hg^{2+}, and Mg^{2+}, where the number following the metal formulas indicates the charge on the metal. A plus charge represents how many electrons have been removed from the metal. When a metal has a charge it is referred to as a metal ion. The molecules with which the metal ions interact can be such simple things as water (H_2O) and ammonia (NH_3), or complex molecules such as adenine or even very complex molecules such as enzymes, proteins, and nucleic acids. Dr. Izatt will give specific examples of the types of interactions we are presently investigating with respect to biological molecules. How do we get a solution containing metal ions? A simple example is to dissolve salt in water to obtain Na^+ ions and Cl^- ions in the water solution. Similarly, other metal ions can be obtained by dissolving the proper metal salt. Metals or metal ions are brought into the body through the water we drink (Ca), the food we eat, (Fe), and the air we breathe (Pb). A common misconception is that metal ions float around in solutions or body fluids without being attached to any other molecule. This is not true as metal ions are always attached to one or more molecules. If no other molecules are present, the metal will be attached to water molecules.

Our interest in the interaction of metals with various molecules is to determine two basic properties, the quantities present when the solution is at equilibrium (this property is called the Equilibrium Constant), and the strength of the chemical bonds between the metal and the molecules (this property is called the Heat of Reaction). These two properties are basic in understanding how metals react with molecules. The extent of a reaction can be visualized by looking at the reaction between

hemoglobin and oxygen (Figure 1). I have written the re-action using two different nomenclatures to show how hemo-globin in the blood combines with oxygen in the lungs and carries this oxygen to other parts of the body. (Remember story number 2 about the track athletes.) Hemoglobin contains iron and it is the iron that reacts with oxygen. A solution of hemoglobin and oxygen can be described by an equilibrium constant, K

$$K = \frac{(\text{Amount Hemoglobin - Fe - } O_2)}{(\text{Amount Hemoglobin - Fe}) \ (\text{Amount } O_2)}.$$

If hemoglobin and oxygen are mixed some of the hemoglobin reacts with the oxygen while some does not. The equilibrium constant is the ratio of the amount of the (hemoglobin-Fe-O_2) to the amounts of the (hemoglobin-Fe) and (O_2). At equi-librium all three of these molecules will be present.

By knowing the value of the equilibrium constant, we can predict how much of the hemoglobin will react with oxygen for any situation. For example, in the case of the athletes breathing carbon monoxide, if the equilibrium constants for the reaction of oxygen and carbon monoxide with hemoglobin are known, it is possible to calculate how much of the hemo-globin is combined with oxygen and how much is combined with carbon monoxide.
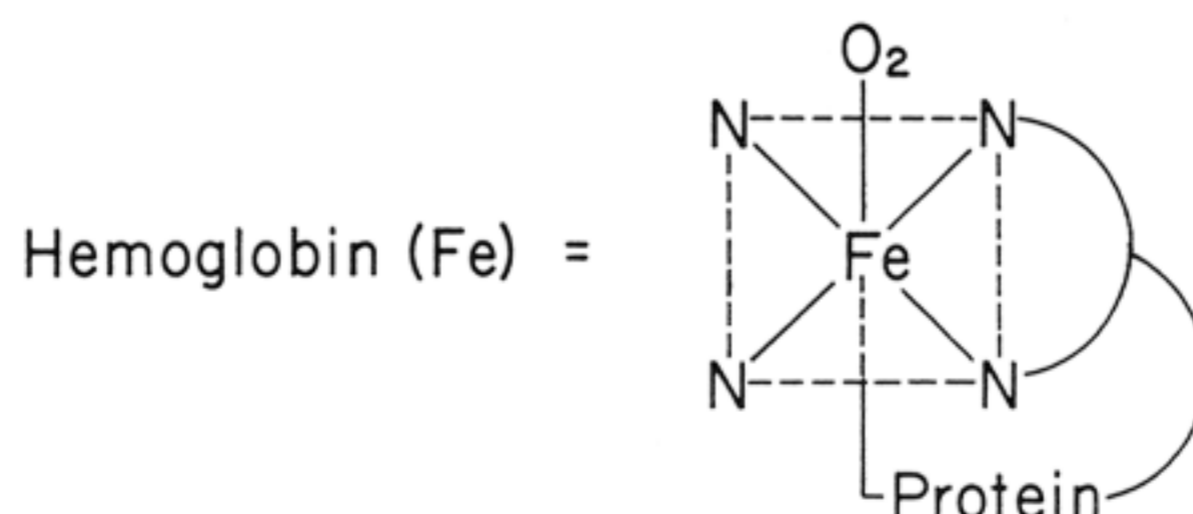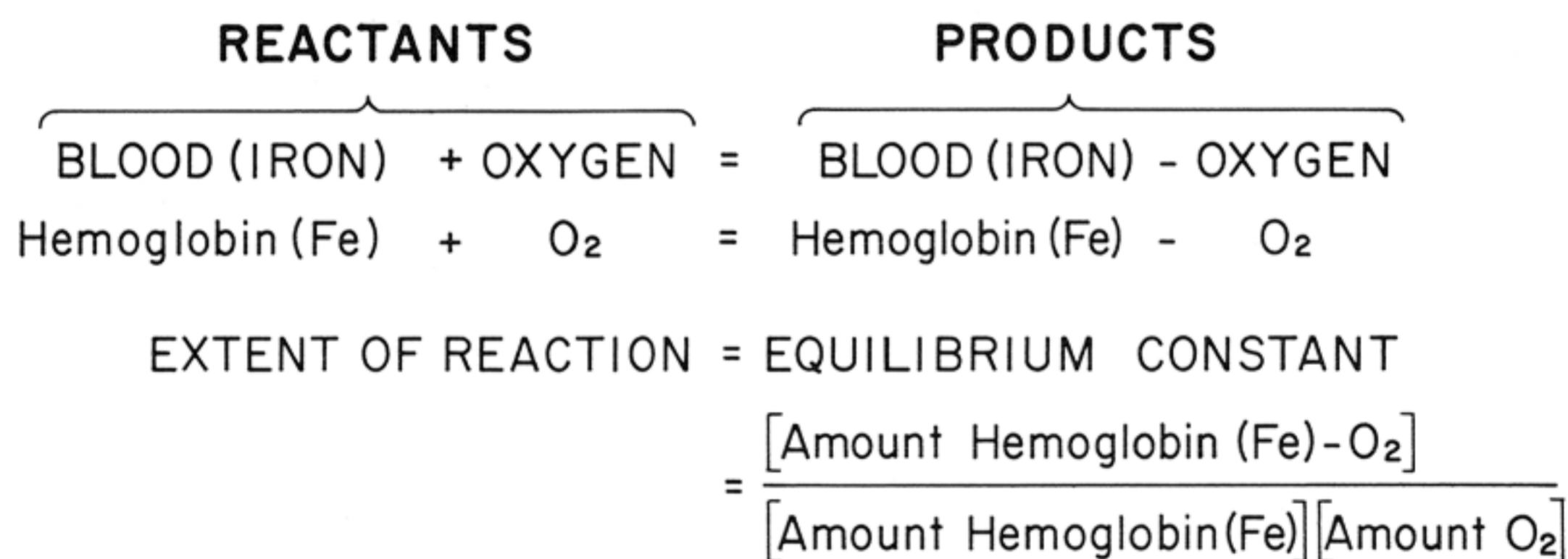
REACTANTS   **PRODUCTS**

BLOOD (IRON)   + OXYGEN   =   BLOOD (IRON) - OXYGEN

Hemoglobin (Fe)   + O_2 =   Hemoglobin (Fe) - O_2

EXTENT OF REACTION = EQUILIBRIUM CONSTANT

$$= \frac{[\text{Amount Hemoglobin (Fe)-}O_2]}{[\text{Amount Hemoglobin(Fe)}][\text{Amount } O_2]}$$

Hemoglobin (Fe) =

Figure 1

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The other basic property we wish to know is the heat of reaction. When a molecule is formed, chemical bonds are made, or when a molecule breaks apart, chemical bonds are broken. The strength of chemical bonds tells us much about the type of bonding between the atoms in the molecule and the energy released or absorbed when changes occur. One example of breaking bonds is when natural gas is burned in a furnace (Figure 2). The following reaction occurs:

$$CH_4(gas) + 2O_2(gas) = CO_2(gas) + 2H_2O(liquid)$$

resulting in the breaking of six bonds (4 for CH_4 and 2 for $2O_2$) and the making of 6 bonds (2 for CO and 2 for $2H_2O$)

REACTANTS **PRODUCTS**

METHANE + OXYGEN → CARBON DIOXIDE + WATER
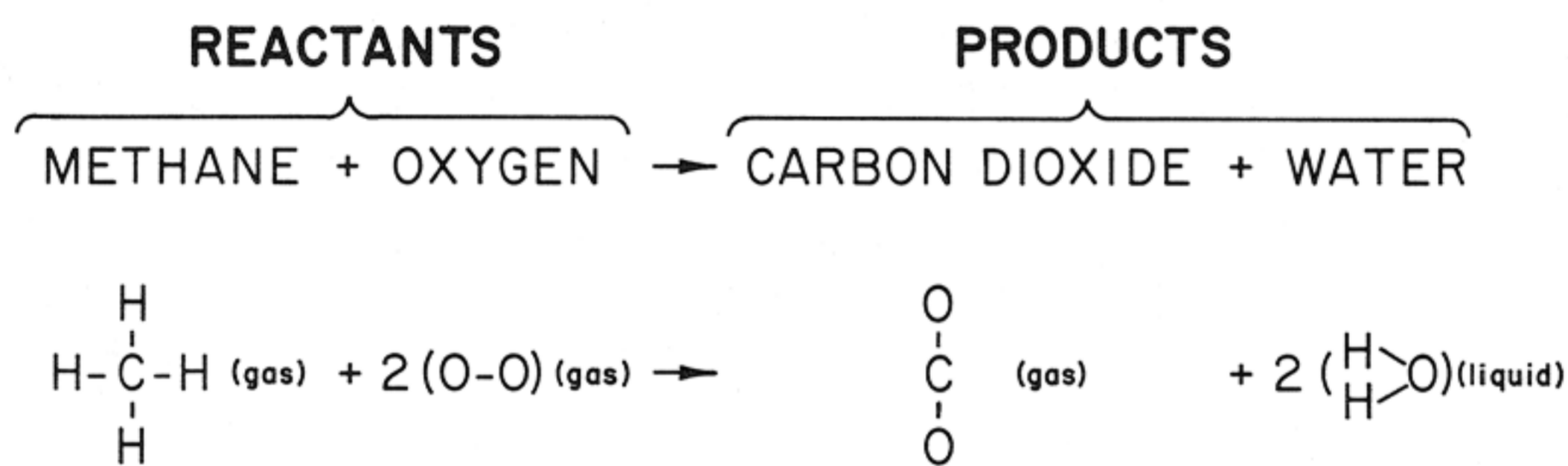
HEAT RELEASED = 212,800 calories/
16 grams methane

Figure 2

and the liberation of 212,800 calories of heat per 16 gm. of methane burned. This 212,800 calories is the heat of reaction. This example shows that chemical bonds are not equal in that the same number were made as were broken, but energy was released. In combustion, this energy heats up the CO_2 and H_2O to give the hot flames we all observe and the warm houses we all enjoy. A calorie as used here is defined as the energy required to raise the temperature of 1 gm. of water 1°C. They are 1/1000 the calories used in nutrition and by calorie counters. An example of the extent and chemical energy of reaction is the reaction of adenosine triphosphate with water to release energy.
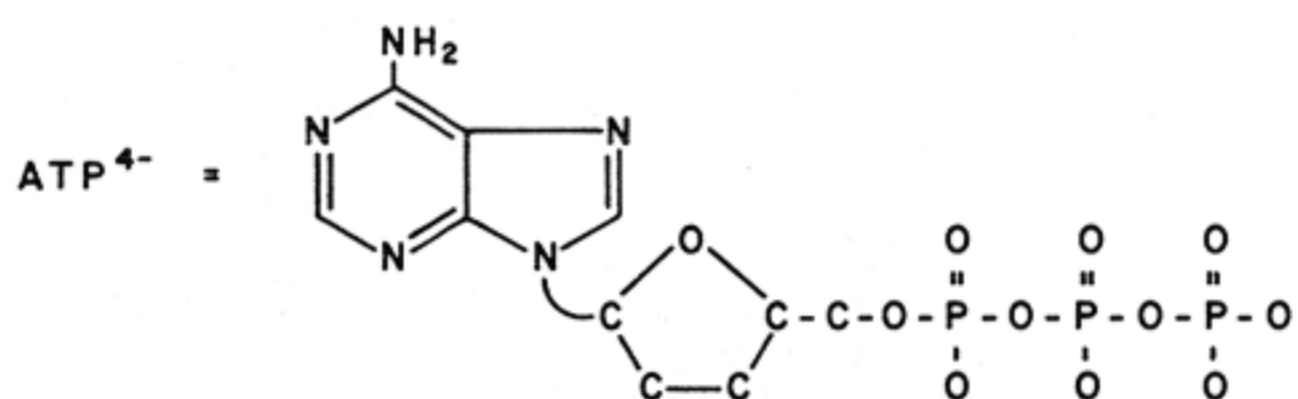
$$ATP^{4-} + H_2O = ADP^{2-} + HPO_4^{2-}$$
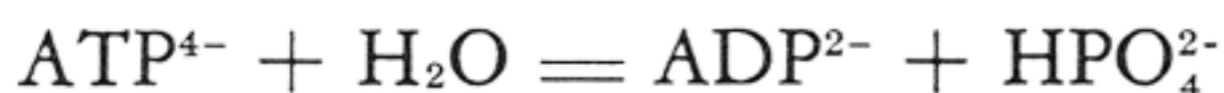
Adenosine triphosphate (ATP) is the molecule shown in Figure 3. It is in this molecule that the body stores energy that will be

ADENOSINE TRIPHOSPHATE + WATER = ADENOSINE DIPHOSPHATE + MONOHYDROGEN PHOSPHATE

$$ATP^{4-} \quad + H_2O = \quad ADP^{2-} \quad + \quad HPO_4^{2-}$$

HEAT RELEASED = 7,000 calories

14.6 calories = 174 followed by 19 zeros molecules

Figure 3

used for many of the energy consuming processes taking place in the body. The net energy release in breaking and making bonds by this reaction is 7,000 calories per 450 grams ATP. This energy is available for other reactions. One example of this is the energy used in muscle contraction which is derived from ATP bond breaking (*Scientific American,* April 1970). In lifting a 15 lb. child three feet a parent does

$$15 \text{ lb. } (3\text{ft}) = 45 \text{ ft. lb.} \frac{(0.324 \text{ cal.})}{\text{ft. lb.}} = 14.6 \text{ calories work}$$
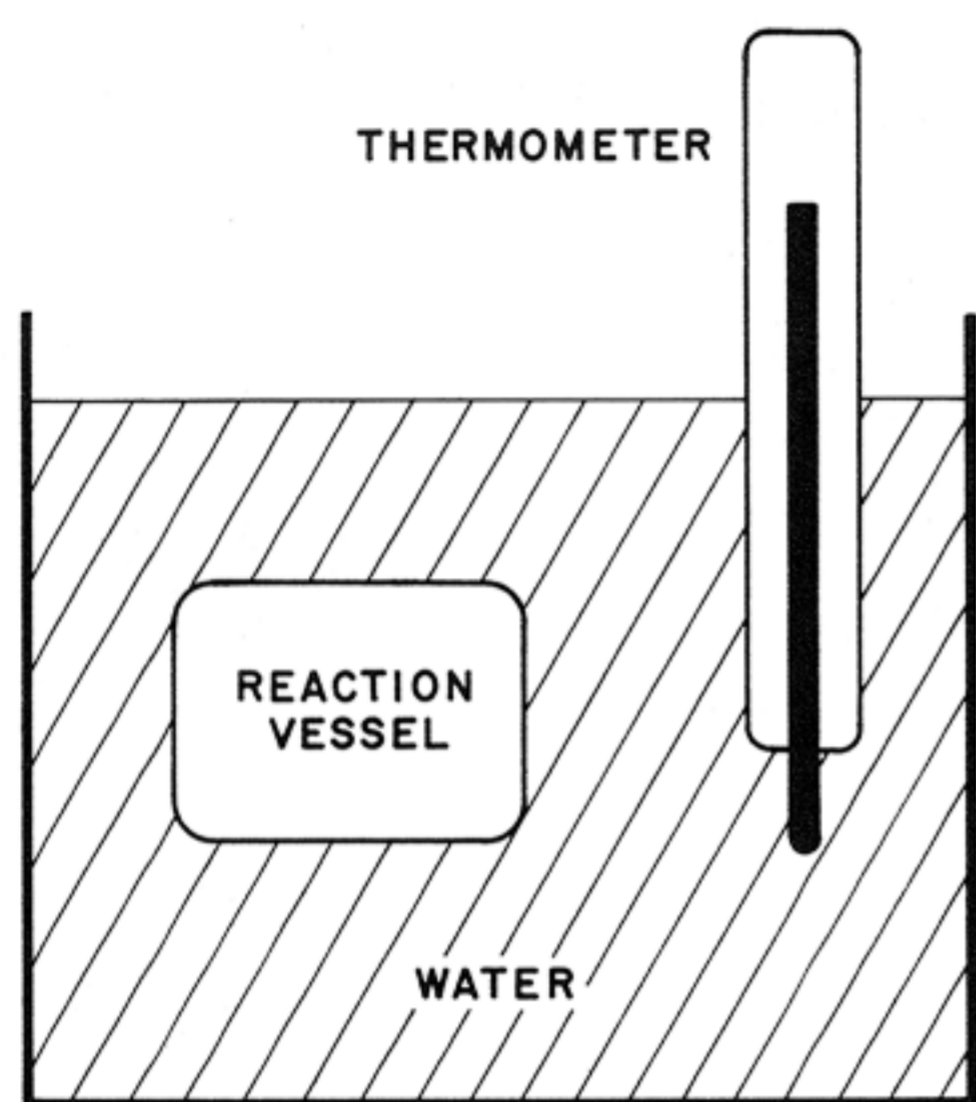
This requires the breaking of

$$\frac{6.023 \times 10^{23} \text{ bonds } (14.6 \text{ cal.})}{7,000 \text{ calories}} = 1.74 \times 10^{21} \text{ bonds or molecules}$$

of ATP which is the number 174 followed by 19 zeros. The equilibrium constant tells us how much ATP is present for any condition and the heat reaction indicates the energy released or absorbed when a change takes place.

To understand the interactions of metals and molecules in solution, it is necessary to know values for the equilibrium constant and the heat of reaction. Quantities of heat and, therefore, heats of reaction can be measured experimentally in calorimeters. We have found that by the use of certain novel and sophisticated equipment and procedures developed in our laboratories, that equilibrium constants, as well as heats of reaction, can be measured calorimetrically. Thus, one experi-

ment can yield all the necessary data, resulting in a great savings in time and effort.

Let us now look at some calorimeters in which heats of reactions can be measured. A conceptual diagram of a calorimeter for measuring heats of combustion is shown in Figure 4. The main components of the calorimeter illustrated here are the *reaction* vessel, where the reaction occurs, the *surrounding water* and the *thermometer*. The heat generated in the reaction vessel flows into the surrounding water, heating it, and causing its temperature to rise. An experimental run consists of initiating a reaction in the reaction vessel and measuring the temperature rise of the water. A similar experiment where a reaction releases a known amount of heat serves to calibrate the calorimeter in that the amount of heat required to produce a given temperature increase is determined. A calorimeter of this type would be used to calculate the heat released for the reaction

REACTION

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O$$

Figure 4

$$CH_4 + 2O_2 = CO_2 + 2H_2O.$$
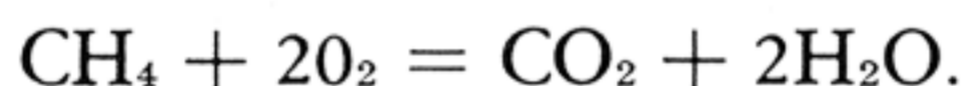
It is interesting to note that the name calorimeter is derived from a combination of the words calorie and meter. The name calorie is derived from the Latin word calor, meaning heat. Up to the early 1800's it was believed that heat was a fluid called caloric and that caloric was contained in materials much as water can be contained in a water pitcher. Caloric was defined as being an elastic fluid of great subtility, the particles of which repel one another, but are attracted by all other bodies. The force of attraction was directly related to the temperature difference between the two bodies. We now know, thanks initially to the work of Count Rumford (Benjamin Thompson, 1753-1814) in cannon-boring experiments carried out in the Munich Arsenal in 1790 (Figure 5), that heat is not a fluid
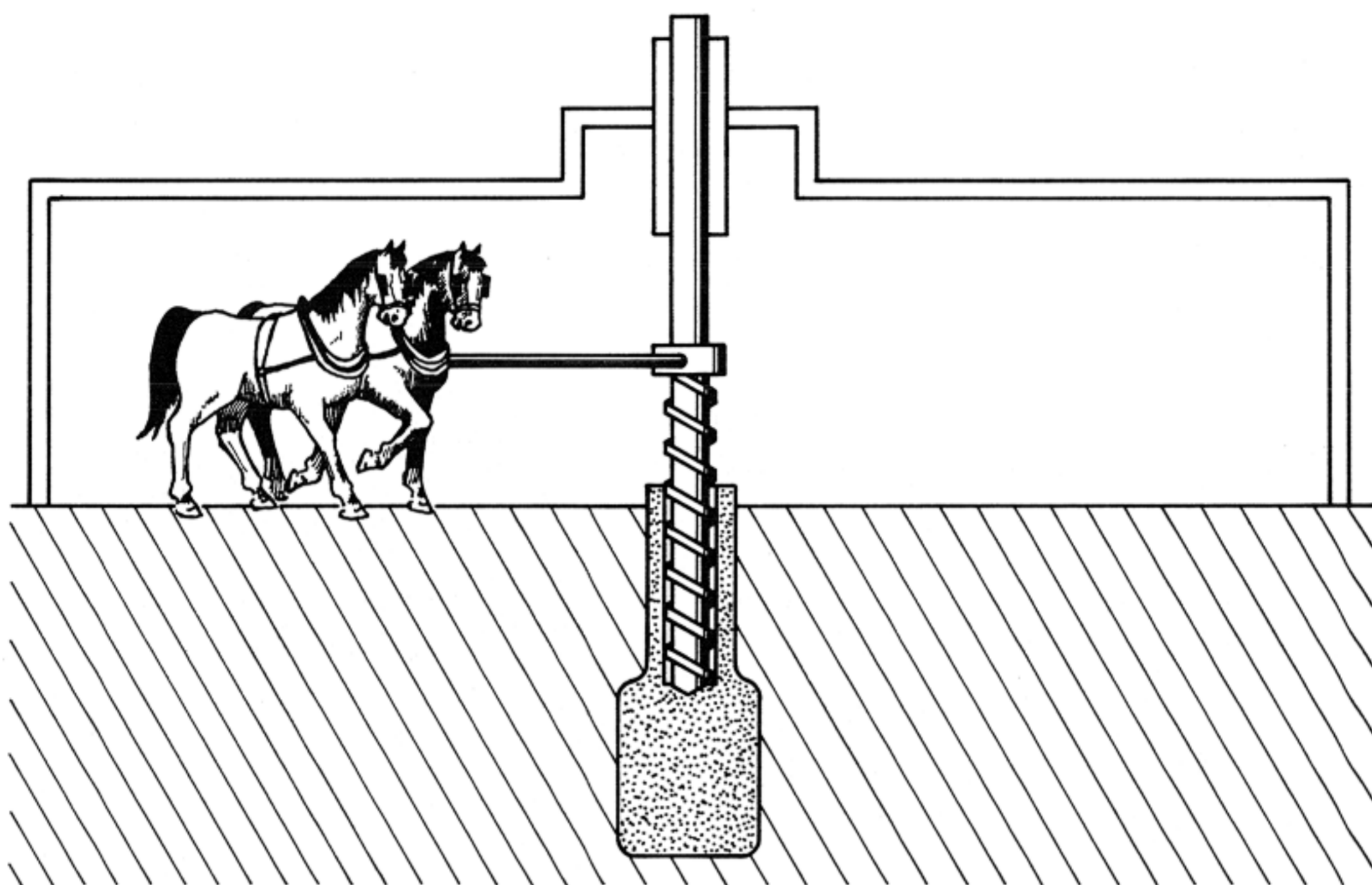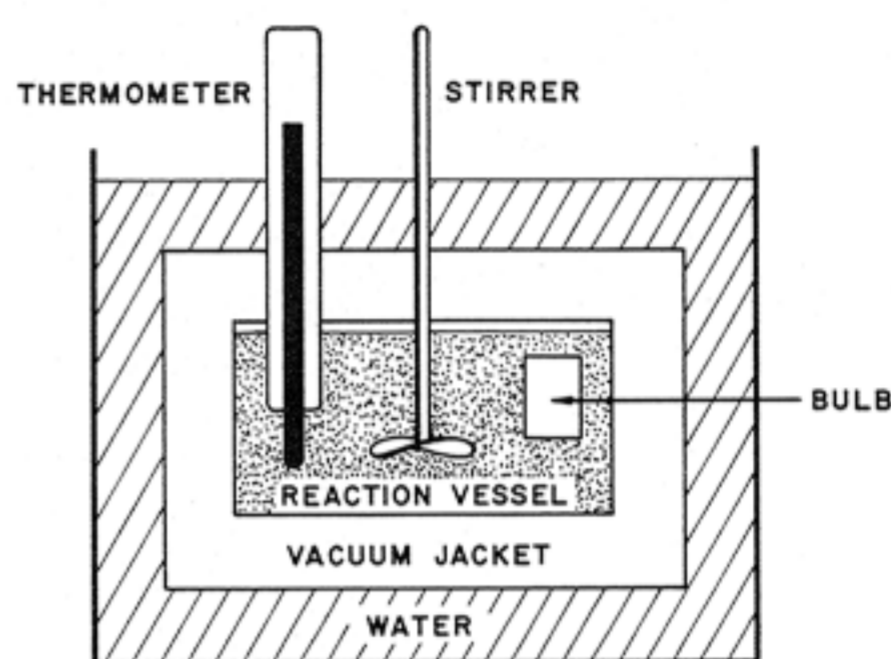
Figure 5

but is energy and is stored in molecules and atoms in the form of bond energies and molecular motion. Rumford showed in his experiments that heat could be produced without limit and thus cannot possibly be a material substance. The situation would be like pouring water without ceasing from the pitcher which is impossible if water is a material substance. However, Rumford's results have also brought some discomfort to teachers of thermodynamics. They frequently describe in class Rumford's cannon-boring experiment in which he employed a team of horses to turn the drill bit in the cannon muzzle. Those who pose the question, "Now what quantity is produced without limit in this experiment?" always receive the shouted answer, "Horse manure."

It is precisely by this method of calorimetry that the calorie content for foods is determined. The body takes food through a series of reactions involving many bond breaking and bond making steps and ultimately reduces the food to the same product as would result from the reaction of the food with oxygen in the calorimeter i.e., CO_2 and H_2O, and liberating energy in the process. So, in a sense when you eat you are not eating calories, but chemical bonds. Fat is just an efficient way of storing the bonds that are not broken.

Reactions occurring in the solution usually have much

smaller energy changes than those for the combustion of gases. A conceptual diagram of a conventional solution calorimeter for measuring heats of reaction in solution is shown in Figure 6. The main components of the calorimeter illustrated here are the reaction vessel containing a bulb, a thermometer and a stirrer; the vacuum jacket on the reaction vessel and the surrounding water. This calorimeter differs from the previous one in that a vacuum jacket reduces the flow of heat from the vessel to the water. If the heat of reaction were allowed to heat the surrounding water, very little temperature rise

REACTION

Hemoglobin (Fe) + O_2 = Hemoglobin (Fe) - O_2

Figure 6

would be noted due to the small heats involved. Therefore, the heat is contained and the temperature rise of the solution in the reaction vessel is measured.
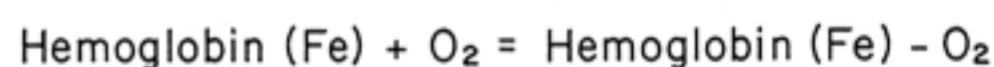
The materials to be reacted are kept separated by placing one in the bulb contained in the reaction vessel while the other one is in the solution surrounding the bulb. The operation of this calorimeter can be illustrated by considering the formation of a complex between hemoglobin and oxygen. The hemoglobin is in a diluted solution in the reaction vessel and the oxygen is contained in the bulb. The bulb is broken and the solutions mixed with a resulting temperature rise, measured by the thermometer in the solution. A similar experiment using a substance with a known heat of reaction will give the heat required to produce a given temperature increase.

Now, as I said previously, we have developed novel equipment and calculation procedures to where we are able to calculate both equilibrium constants and reaction energies from calorimetric data. The technique we use is called titration calorimetry and involves the addition of one of the reactants over a period of time to the reaction vessel containing the other reactant. A conceptual diagram of a titration calorimeter is shown in Figure 7. The main components of the calorimeter illustrated here are the reaction vessel containing a thermometer, stirrer, and titrant tube; a vacuum jacket on the reacting

REACTION

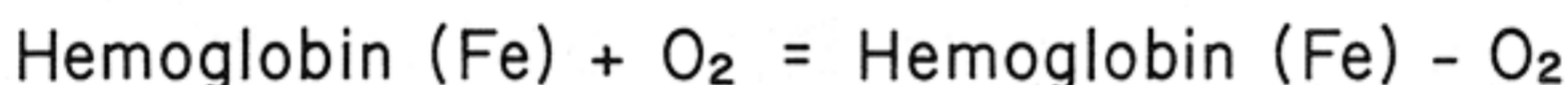Hemoglobin (Fe) + O_2 = Hemoglobin (Fe) – O_2
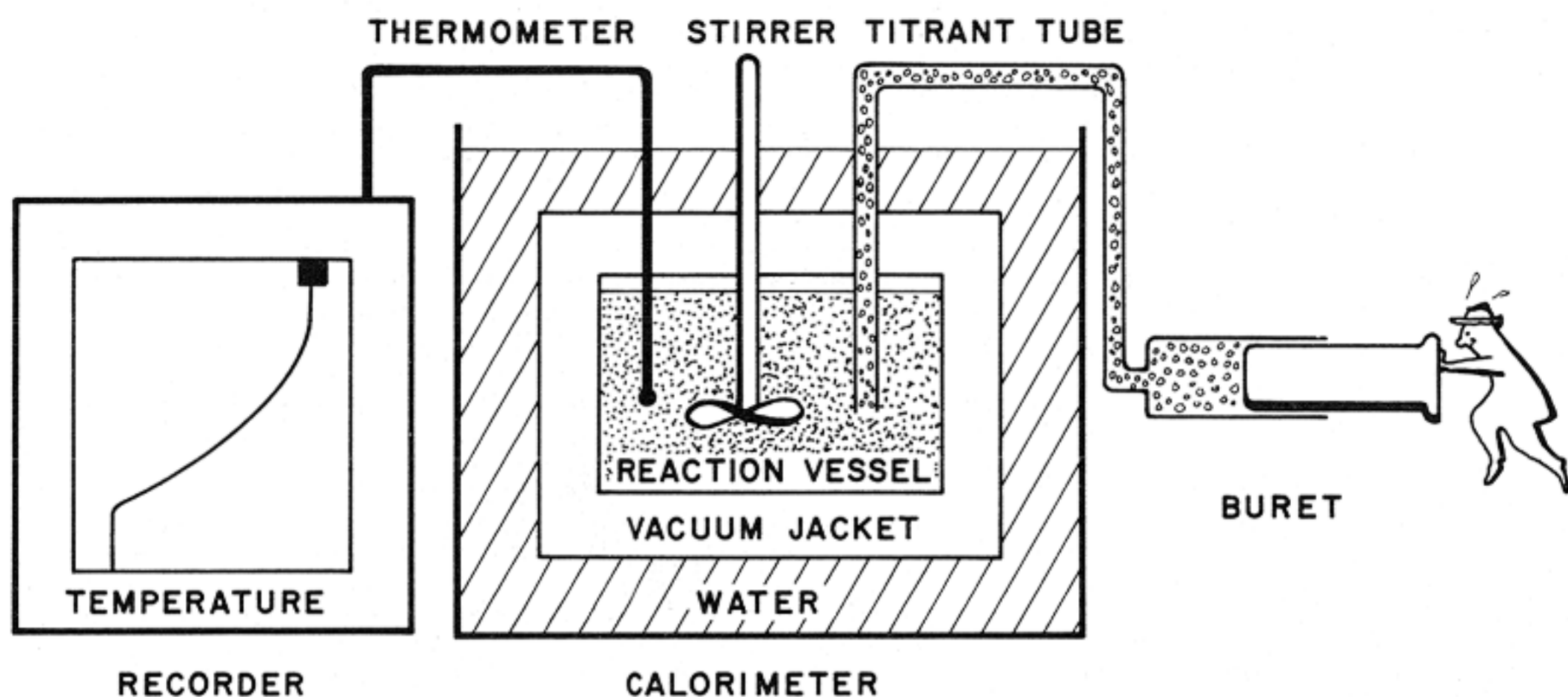
Figure 7

vessel; the surrounding water bath; recorder and buret. The temperature is sensed by a rapid reading thermistor and recorded on a strip chart recorder. This calorimeter differs from the other previously shown in that one of the reactants is introduced continuously over a predetermined length of time and the temperature is recorded. The chemical system hemoglobin-oxygen can again be used to illustrate the operation of the equipment. With this calorimeter, however, it is possible to calculate not only a heat of reaction but an equilibrium constant as well. This reaction would be carried out by placing a hemoglobin solution in the reaction vessel and titrating with oxygen gas. It should be noted that by titrating with oxygen the reaction can be stretched out over any chosen time length in contrast to the method of breaking a bulb containing the oxygen in which the reaction takes place immediately. Each small addition of titrant is thus equivalent to a separate experiment using the bulb breaking method. Also, the shape of the temperature curve is determined by both the heat of reaction and the extent of reaction. From one experiment by certain techniques, many of which were developed here at BYU, the equilibrium constant and the heat of reaction for the hemoglobin-oxygen reaction can be determined.

Equipment of the type visualized here and based on concepts developed at BYU is currently being manufactured in the Provo-Orem area by Tronac, Inc. A picture of this com-

mercial unit is shown in Figure 8. This equipment retails for the price of 9 to 12 Maverik automobiles ($18,000-24,000) but considering how much more it can do than a Maverik it is a real bargain at this price.

Equilibrium constant and heat of reaction values represent a window through which one can see and interpret metal-molecule interactions. These numbers can be used to aid in the solution of problems in such widely diversified fields as Saline water conversion and the recovery of minerals from the sea to nerve impulses and the operation of the brain.

In these and other areas it is important to know how much of the metal ion is reacted with the other molecules and how much energy is released or absorbed when reaction occurs. We can see how calorimetry can be a powerful tool for solving problems concerning metal binding in biology.

Figure 8

Now we have spent some time describing how certain measurements are made and it would perhaps be well to ask what metals occur in biological systems and in what form and concentration. A few of the more common metals and where they occur are: calcium, bone; iron, hemoglobin; cobalt, vitamin B12; magnesium, chlorophyll and sodium, human blood. A very large number of metals is present in even a single strand of human hair (*viz.,* silver, iron, copper, zinc, manganese, strontium, boron, cobalt, lead, aluminum, magnesium, calcium, silicon, titanium, nickel and chromium. In fact, probably every metal known to exist is present in the body—some at a much higher concentration than others. Metals, specifically sodium and potassium take part of the chemistry of nerves and supply the electrical charges necessary for the transmission of impulses along nerves. Metals are also connected with enzymes—those molecules that make reactions occur in living systems. However, I am now getting into an area of describing how metals take part in biological phenomena. I would therefore at this time like to turn the lecture over to Dr. Izatt who will describe in detail the work in which we are currently engaged in the field of the transport of metal ions across membranes, and the chemistry of nerve action.