HIGH PRESSURE REACTION VESSEL SYSTEM I. INSTALLATION, MODIFICATION AND INITIAL EXPERIMENTS

by

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SUMMARY PAGE

THE PROBLEM

To modify an existing high pressure vessel system for the satisfactory analysis of reactions carried out in metabolizing biological systems under conditions simulating pressures down to 1,000 feet of sea water.

FINDINGS

A series of modifications was developed for precise control of reaction conditions and times to safely allow injection of reaction components into the pressure vessels after pressurization. Additional modifications were also required and devised for enabling rapid and efficient cleaning of reaction vessels and attendant components. Appropriate sampling devices have been fabricated and installed to allow analyses of products of reaction mixtures by standard laboratory methods such as gas chromatography, spectrophotometry, and radioisotopic analysis.

APPLICATIONS

In order to properly understand the biological effects of high pressures on human tissues it is necessary to be able to study simplified biological systems exposed to increased pressures under laboratory conditions. The system that we now have available allows observations on the influence of varying pressures or temperatures or varying solution components on tissue preparations from animals, on appropriate human tissues such as blood or on single-celled organisms.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Independent (In-House) Work Unit MR011.01-5051 -- Installation, Modification and Feasibility Testing of High-Pressure Metabolic Reaction System. The present report is the final report on this work unit. The manuscript was approved for publication on 9 June 1972. It has been designated as Naval Submarine Medical Research Laboratory Report Number 713.

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ABSTRACT

A high pressure system equipped with several small sample vessels was installed in the Biochemistry Laboratory at the Naval Submarine Medical Research Laboratory and successfully modified for conducting a variety of biological reactions. Various problems encountered in making modifications to the system and their solutions are described. The most successful experiments to date have utilized erythrocytes and liver preparations for the study of glycolysis under various atmospheres and pressures. The principal advantages of the system as it is being developed is its versatility in reaction conditions and methods of sampling that can be produced and utilized.

HIGH PRESSURE REACTION VESSEL SYSTEM
I. INSTALLATION, MODIFICATION AND INITIAL EXPERIMENTS

INTRODUCTION

The basic hardware of the equipment which will be discussed was developed at the University of Florida under ONR contract number 411 and NONR number 580 and has been described in a report by Garrett and Smith[1]. The original purpose of the apparatus was to measure the solubility of gases in biological fluids and tissues. The system, partially depicted in Figure 1, was transferred to the Submarine Medical Research Laboratory in June of 1971 and was modified in its configurational layout to meet the requirements of our existing laboratory facilities. Studies involving equipment with capabilities similar to those of the apparatus to be described have been reported by other workers.[2-4]

As initial stages of our work progressed, it became increasingly obvious that, although the existing device formed a very good basic building block, several rather extensive modifications would be necessary to allow its use in proposed biochemical experiments. These modifications have included changes in mechanical and fluidic control devices, in the physical aspects of sample handling, and in the electronic control system.

Equipment Modification

One of our earliest and most obvious problems was the need for a method whereby reaction components could be added to pressure vessels to initiate or modify a biochemical process after the system was pressurized. Three of the reaction vessels were modified to allow this to be done. Figure 2 illustrates the point at which the necessary changes were made while Figure 3 depicts the actual modification assembly.

The valve and sample cylinder assembly shown in Figure 3, operating in conjunction with existing pressure vessel controls, allows the injection of necessary reaction components from the sample cylinder. This is done by creating a small pressure differential of short duration over the injectate sufficient to inject the reaction component into the pre-pressurized reaction vessel.

Another troublesome requirement imposed by the use of the equipment for biological reactions was a method for cleaning the cells without resorting to dismantling the entire assembly. This was ultimately accomplished by replacing the drain plug of the lower block assembly shown in Figure 4 with a modified ball valve and check valve assembly. Necessary tubing was then installed to allow the content of the pressure vessels to be blown into a waste collection reservoir.

After making the above modifications to the lower block assembly it was found

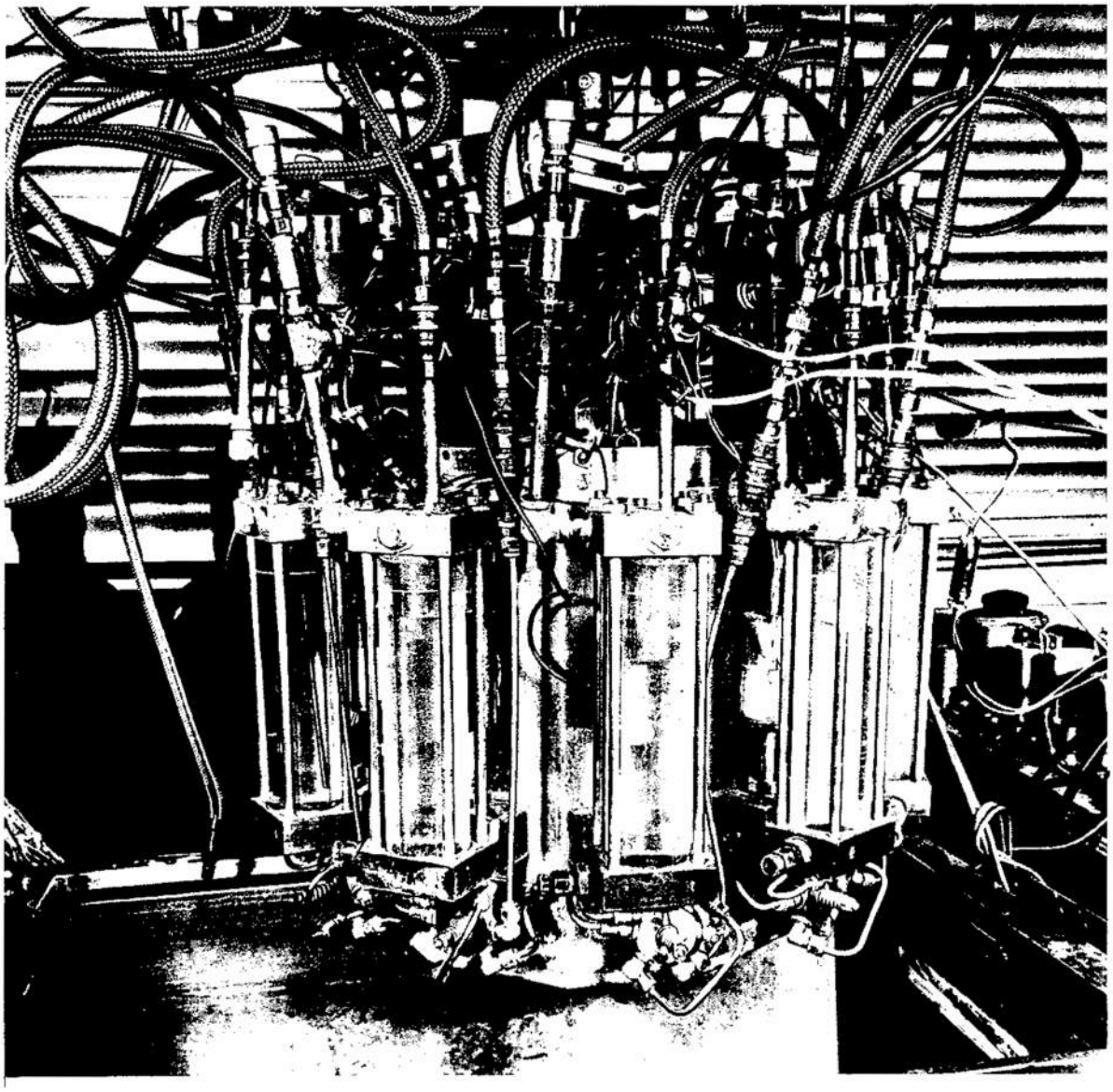
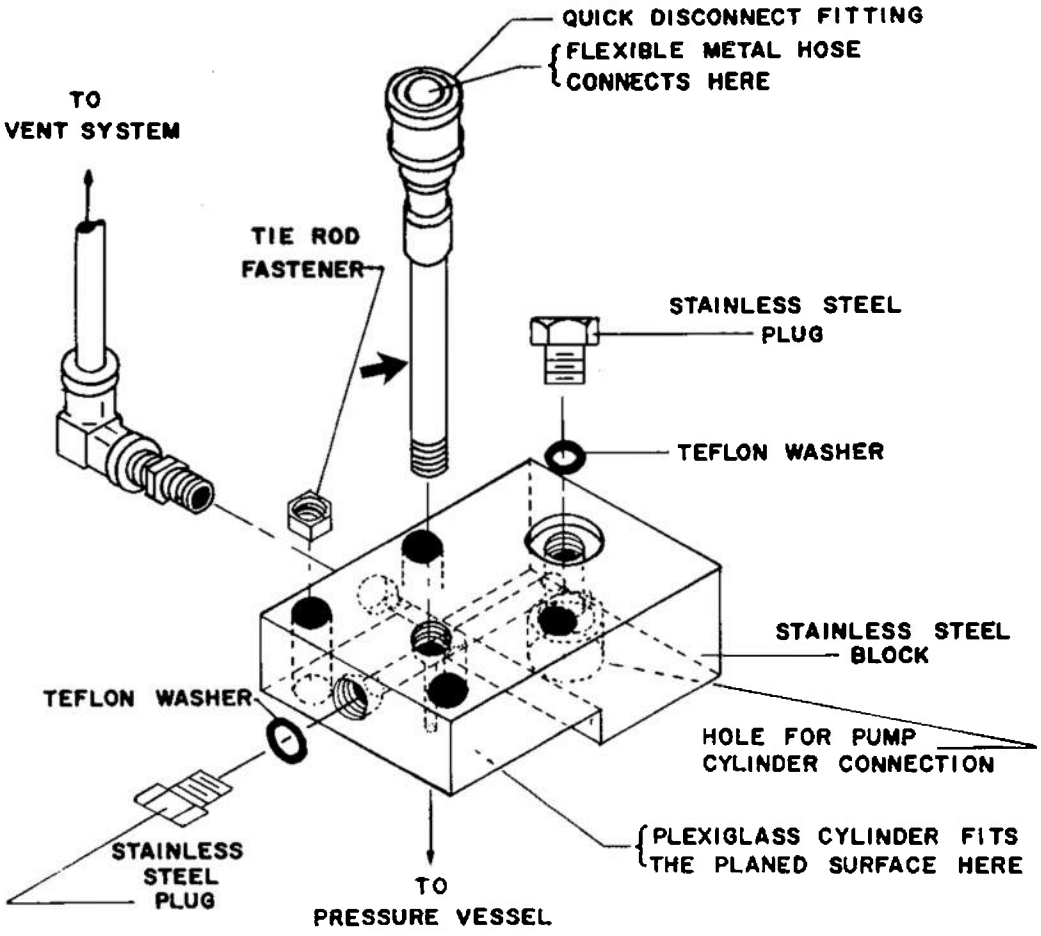


Fig. 1. Depiction of the dry well assembly with its attendant reaction vessels.

that the addition of cleaning fluids to the reaction vessels was still a laborious and time consuming process. To circumvent this problem a pressurized cleaning system was designed and constructed utilizing a pressure tank, which was connected through necessary electrically controlled valves by a flexible metal hose. With these latter two modifications it is now possible to rapidly and efficiently clean both the pressure vessels and the attendant reaction component loading systems.



TOP BLOCK ASSEMBLY

Fig. 2. Large arrow indicates the section replaced by the modification shown in figure 3.

The original high pressure reaction device utilized a liquid injection system to transfer an aliquot of sample solution to the sparging chamber (a specialized chamber where a carrier gas is bubbled through the liquid sample to remove the equilabrated gas, see Figure 5). Subsequently it became apparent that some method for transfer must be developed that would not dilute either the individual aliquots removed or the remaining reaction mixture in the pressure vessels. After several attempts to arrive at a satisfactory design, a successful gas injection system was devised and installed. A test control circuit was a necessary prerequisite for the development of such a workable system.

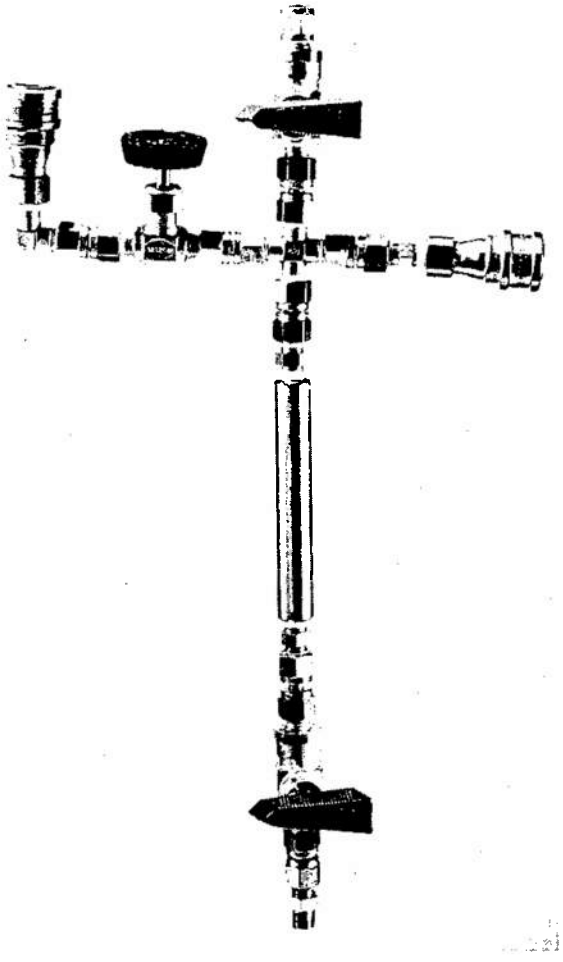


Fig. 3. Representation of modified sub-assembly used to replace section of the top block assembly indicated in figure 2.

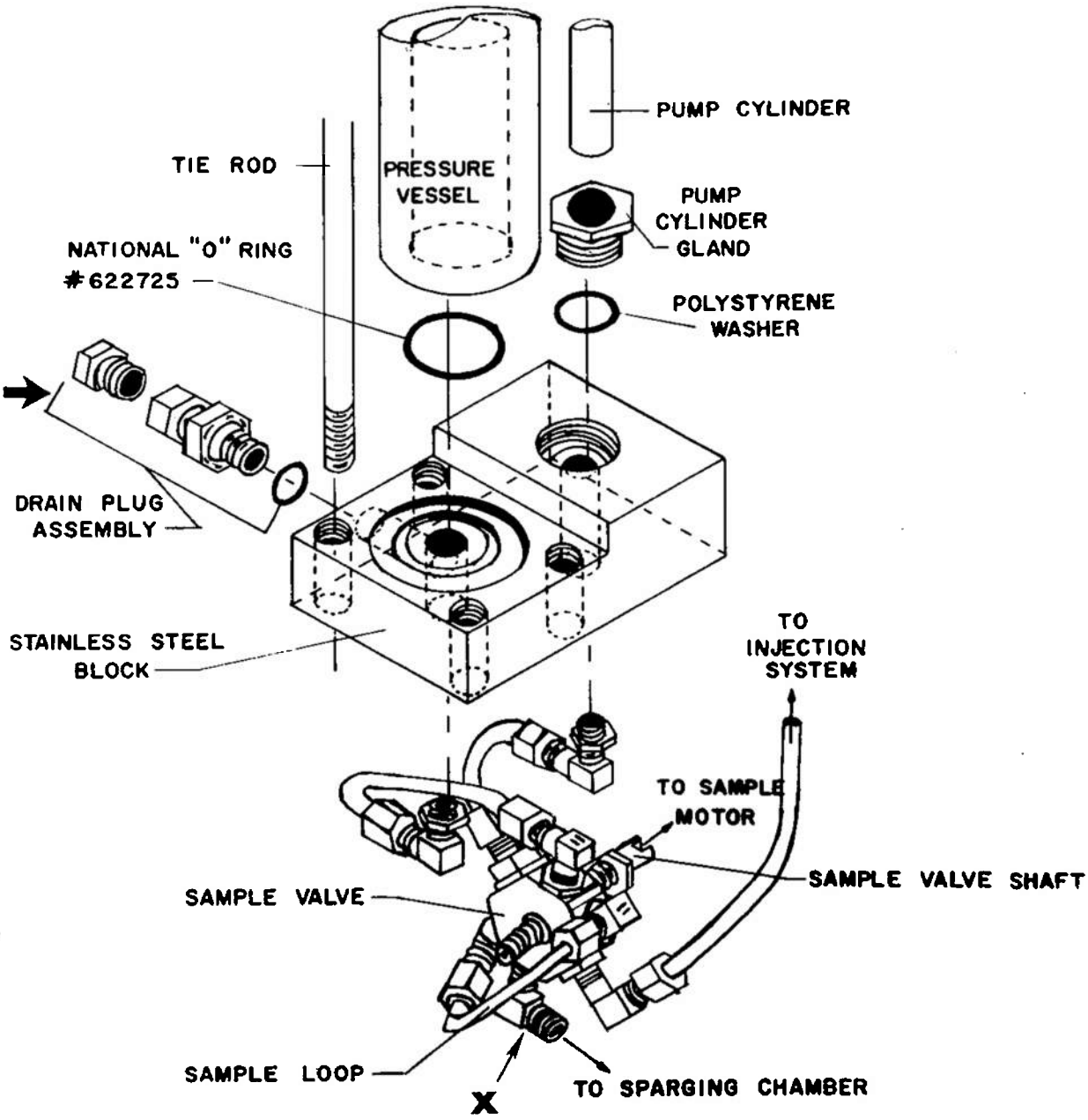
Circuit changes and new circuit designs for integrating the gas injection system into the principal control system were required after completion of the modifications.

The use of the gas injection system has solved the problem of undiluted sample transfer and since the injection gas used is the same as that used to pressurize the reaction vessel, there is no gaseous contamination of the reaction mixture due to trapping in the sample loop.

A method for stopping the chemical reactions in the sample immediately after depressurization led to the next modification which was to the sparging chamber itself. Figure 5 illustrates the point at which this modification was introduced. The existing cap and elbow were removed and a system consisting of solenoid valves, a ball valve, a stainless steel sample cylinder, and necessary fittings was connected at the point indicated. The required electronic controls were designed and installed to allow automatic injection of controlled quantities of an inhibiting agent into the sparging chamber at the appropriate times.

Originally the sparging chamber could be cleaned by using a flush gas only. For performing biochemical experiments it was deemed necessary to introduce additional liquid flushes to avoid contamination of succeeding samples. To do this a system of valves and fittings was used to connect the pressurized wash reservoir with the sparging chamber at the point where injection of the inhibiting agent takes place. An additional electronic control system was therefore designed and constructed to control the flow of wash liquid into the chamber. This system was integrated into the existing control circuits so that the sparging chamber is now subjected to three repetitions of gas and liquid cleaning between each sample.

A further change made in the high pressure reaction system has been required for conformation with the laboratory's safety standards. The existing hydrogen generator was



BOTTOM BLOCK ASSEMBLY & SAMPLE VALVE

Fig. 4. Heavy arrow indicates the sub-assembly modified for cleaning purposes. Point (X) indicates the site of additional modifications allowing liquid sample collection.

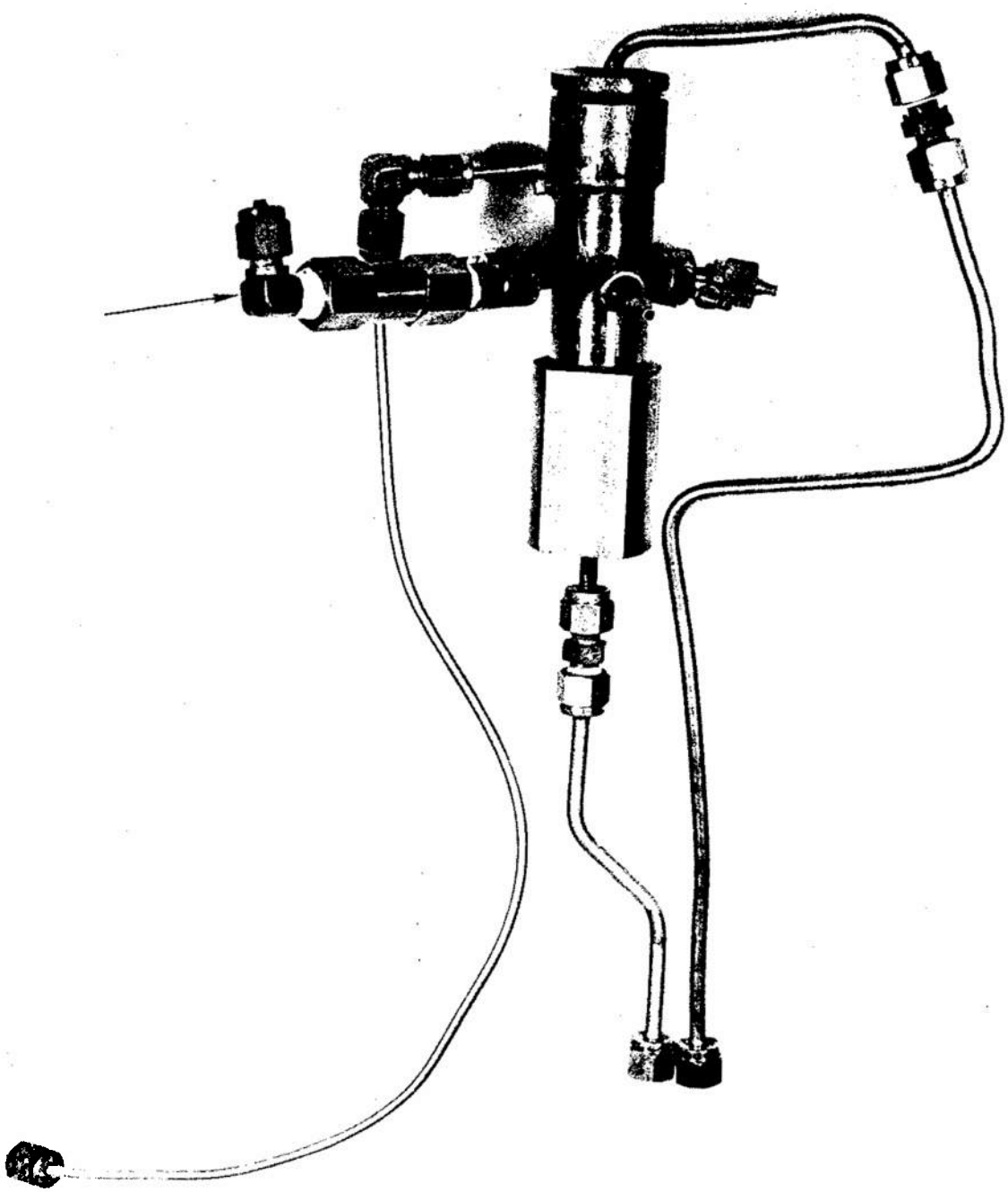


Fig. 5. Representation of sparging chamber with arrow indicating point modified to allow inhibitor and cleaning solution introduction.

removed and the system converted to the use of inert carrier gases. While some changes in the original gas supply lines and gas sources were necessary, no major modifications in the system controls were required by this conversion.

Since some of our proposed experiments require a more rapid sampling rate than permitted by the design of the original device, it has also become necessary to give the high pressure reaction system the capability of a sampling rate on the order of one per minute or less. To do this one of the pressure cell assemblies has been completely redesigned above the lower block. The plexiglass reaction vessel was removed, the lower block was tapped to allow insertion of a fitting, and a stainless steel sample cylinder was inserted as a reaction vessel. Two of the reagent loading assemblies as shown in Figure 3 were used in conjunction with ball valves, solenoid valves, and fittings to allow rapid mixing of the reaction components by spraying them together to start the reactions as they are loaded into the reaction vessel. Two gas supplies are utilized along with appropriate controls to allow maximum flexibility in pressure balancing. This pressure control equipment becomes extremely important when experiments utilizing erythrocytes or other fragile materials are conducted at high pressures.

The above modified portion of the equipment, dubbed the fast reaction system, required several changes in the electronic control circuits to be used in conjunction with the existing control system in order that the various functions of sample isolation, sample transfer, and necessary flushing could be accomplished within the framework of less than one minute sampling intervals. At this point it also became necessary to install a sample loop loading system since the original circulating system, Figures 2 and 4, was no longer part of the design. This, in turn, required further control circuit modifications which were designed, constructed, and installed.

The fast reaction system utilizes, along with a gas injection, the same combination of gas and liquid flush used in the sparging chamber, and has the ability to be cleaned rapidly using the lower block modification and the pressurized wash system described for earlier modifications. Control of the hardware of the fast reaction system may be shifted to the original timing system so that this cell can be used for experiments that do not require short sampling periods as well as for those utilizing the rapid sampling capability.

Trials of the fast reaction system next indicated that the elution time of the gas chromatograph was a limiting factor in the operation of the equipment. In order to overcome this difficulty a change was made in the carrier lines leading to the detector oven. A system of short trap columns was designed and installed. Control of this trap system depends upon the use of electrically operated solenoid valves.

As a consequence of the trap complex, it is now possible to utilize the fast reaction system and retain a series of individual samples until after a run has been completed. The traps are then opened successively with sufficient time between openings to allow the gas chromatograph to properly detect the components of each sample.

After it became necessary to explore the possibilities of using radioactive tracers in some of our experiments, the collecting trap system was modified to allow analysis of radioactive gas by a method other than chromatography. Additional changes were made in the cell cleaning system and the flush lines to better control radioactive solutions and gases. The waste reservoir was shifted into a hood, precautions were taken to make the low pressure lines leak proof, and sample collecting was done inside of the hood. In the work performed to date $^{14}CO_2$ gas has been collected by flushing into alkali traps from the sample collection columns which were filled during the rapid sampling intervals.

In order to avoid certain limitations of gas chromatography and add flexibility to the range of biological measurements, a method of collecting a liquid sample as it is decompressed has also recently been devised and instituted. For this modification a liquid collecting line was inserted at point (X) on Figure 4. As yet no provision has been installed for gradual decompression of samples removed from the reaction vessels although design work on such a change has been initiated. Since considerable technical difficulty has been experienced in obtaining satisfactory analytical data by employment of the obsolete gas chromatrographic equipment now available and since many biological procedures require spectrophotmetric or other methods of analysis, considerable flexibility is acquired by the use of liquid sampling as will be evident from the following section.

Experimental Work

Since the high pressure reaction system arrived at this laboratory equipped with a gas chromatography apparatus, the first rather brief series of biological studies was designed to yield a gaseous product for analysis. Erythrocyte hemolysates were employed as a source of the carbonic anhydrase enzymes and CO_2 production from a buffered bicarbonate mixture was examined.

After learning to separate the carbon dioxide from other gases contained in our pressurizing mixtures, it soon became evident that the amount of product produced was not proportional to the amount of enzyme utilized regardless of the pressure over the reactions. The use of radioactively labeled bicarbonate to produce $^{14}CO_2$ led to further considerations of our ability to measure the rate of the reaction being studied. Since we were not able to continuously remove the product, it became evident that the reaction reached an equilibrium at 30 or 37 degrees before the first sample could be removed and therefore no reaction rate could actually be measured even under 10 atmospheres or more of pressure.

It was necessary to conclude, therefore, that the limits imposed by the valving operations prevented useful studies of carbonic anhydrase even without considering the time required for washes between samples and the analytical delays of the gas chromatograph. The relatively large amounts of gas produced by this reaction could not be utilized to compensate for the inertia of the apparatus.

Subsequent studies for which final results will be presented in a later report, have therefore been restricted to biological processes which are several orders of magnitude slower. To date we have primarily emphasized the study of the glycolytic sequence in erythrocytes. Because of the rate limiting constrictions in this reaction complex, a series of progress evaluations may be made over a period of an hour. Erythrocytic glycolysis was chosen initially because we were working under helium-containing anaerobic atmospheres. Later work has progressed, however, to more biological oxygen-containing atmospheric mixtures and will be extended to nitrogen, nitrogen-oxygen and other combination environments as well as to the use of other tissues.

The ability now available to remove liquid samples from the reaction system provides the opportunity to perform a wide variety of conventional biochemical measurements. From the erythrocyte incubations we have analyzed lactic and pyruvic acid, glucose, 2,3-disphosphoglyceric acid and ATP as indices of glycolysis. A broad spectrum of other metabolic intermediates are candidates for analysis as experiments progress.

Very preliminary studies have been initiated to evaluate glycolytic rate of liver preparations under pressure and considerably more work will be required to adequately compare results of pressure in this more oxidatively oriented tissue with those of erythrocytes which depend heavily upon glycolysis for energy production.

Conclusions

A high pressure system equipped with several small sample vessels has been installed in the Biochemistry Laboratory at the Naval Submarine Medical Research Laboratory and successfully modified for conducting a variety of biological reactions. The most successful experiments to date have utilized erythrocytes and liver preparations for the study of glycolysis under various atmospheres and pressures. The principal advantages of the system as it is being developed is its versality in reaction conditions and methods of sampling that can be produced and utilized.

REFERENCES

1. Garrett, E. R. and A. H. Smith. Solubility of Gases in Biological Fluids and Tissues. Report of ONR Contract 441; NONR: 580; 1971.

2. Hanasono, G. K., J. L. Hollis and S. L. Schwartz. A Pressure Vessel for Biochemical Studies under Hyperbaric Conditions. Anal. Biochem. 34: 470-77, 1970.

3. Fenn, W. O. The Physiological Effects of Hydrostatic Pressures in P. B. Bennett and D. H. Elliot. The Physiology and Medicine of Diving and Compressed Air Work, pp. 37-57, Bailliere, Tindall and Cassell, London, 1969.

4. D'Aoust, B. G. Apparatus for Incubating and Sampling Tissue and for Cell-Free Systems Under Elevated Gas Pressure. Anal. Biochem. 26, 85-91, 1968.

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