

- [54] **AUTO-ANALYTICAL APPARATUS AND ANALYTICAL METHODS**
[75] Inventor: **Clifford Riley**, Burgess Hill, England
[73] Assignee: **The Wellcome Foundation Limited**, London, England
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[51] **Int. Cl.** G01n 31/00, G01n 31/14, G01n 31/16
[58] **Field of Search** 23/253 R, 259, 230 R, 23/230 B (U.S. only) 195/127 (U.S. only)

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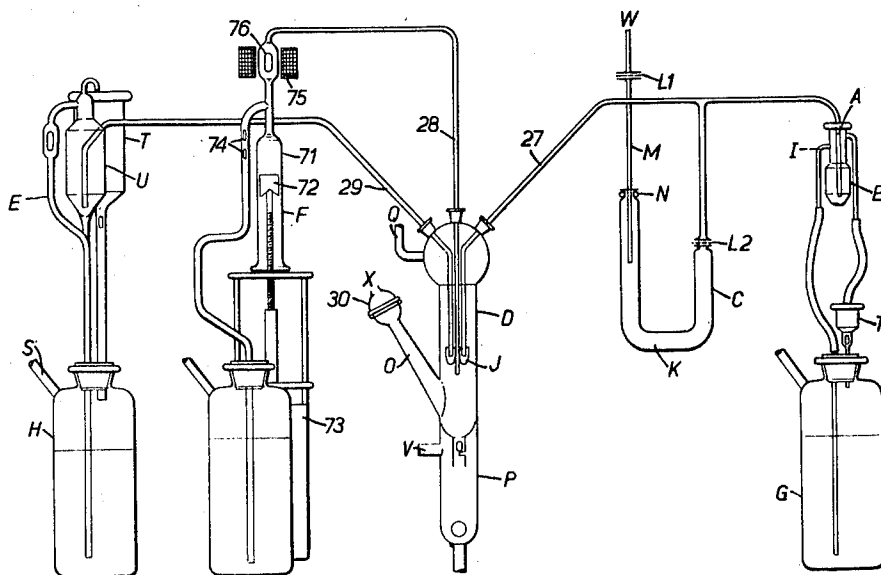
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Primary Examiner—Joseph Scovronek
Attorney, Agent, or Firm—Elliott I. Pollock

[57] **ABSTRACT**

An auto-analytical machine for the analysis of liquid samples comprises, in combination, a reaction chamber, a sample reservoir for holding the sample to be analysed, connecting tubing having one end directed into the reaction chamber and the other end being arranged to be able to dip into a sample in the sample reservoir. A pressure controlled sample measuring device is adapted to move a predetermined quantity of sample into the connecting tubing, and a pressure control mechanism is arranged to move the predetermined quantity of sample into the reaction chamber. The measuring device and the control mechanism are adapted to move the sample by a change of fluid pressure in the connecting tubing. Reagents can be added to the reaction chamber in predetermined quantities, and a titration apparatus can supply a titration reagent at a slow rate until an end point is reached. A method of analysis of a liquid sample can be performed by using the above apparatus to draw a predetermined quantity of the sample into the reaction chamber by changes of fluid pressure so that an analytical reaction may be carried out in the reaction chamber upon the sample.

25 Claims, 17 Drawing Figures



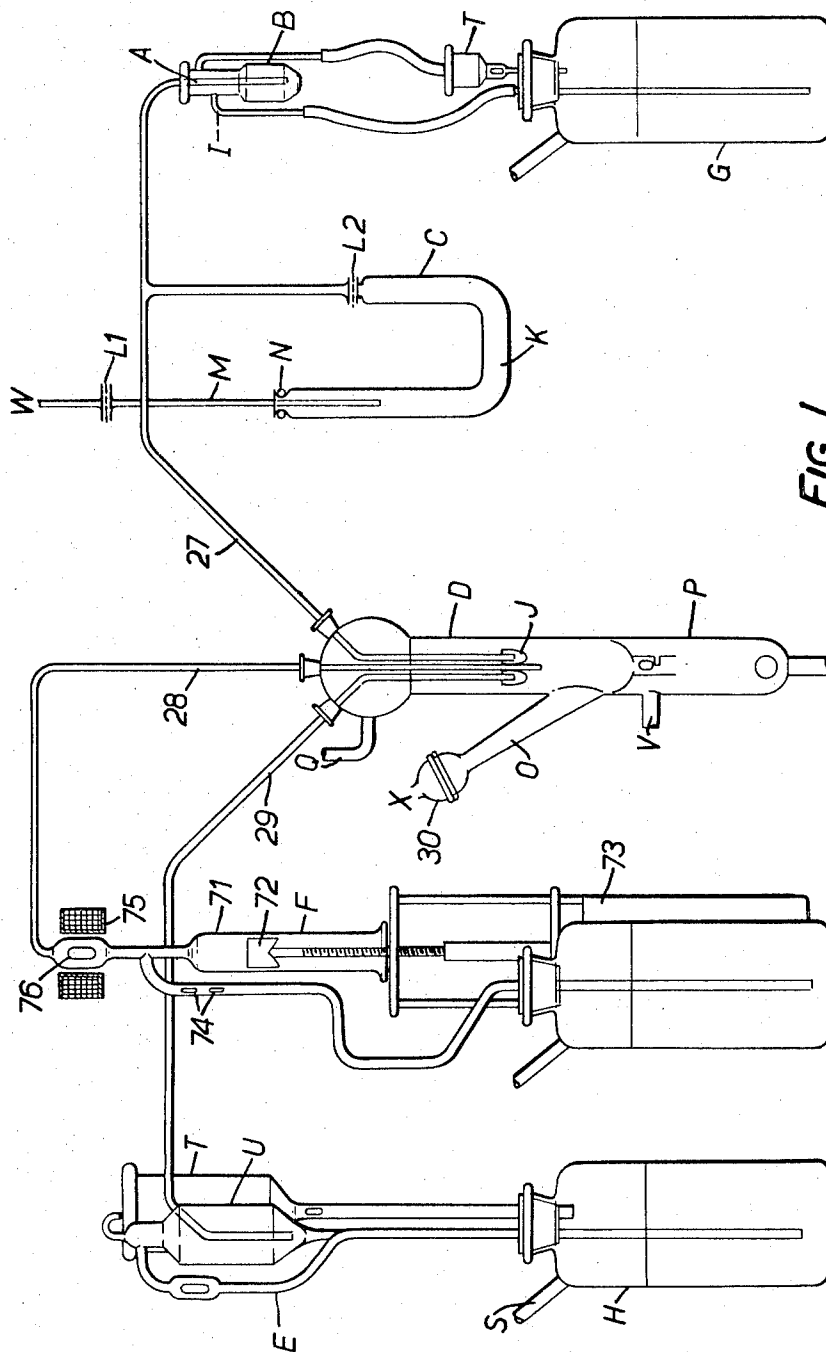


FIG. 1.

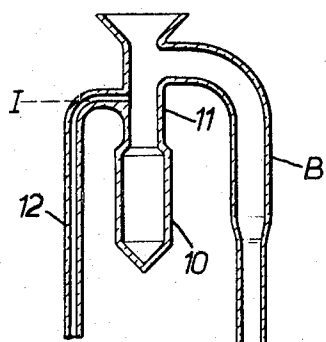


FIG. 2.

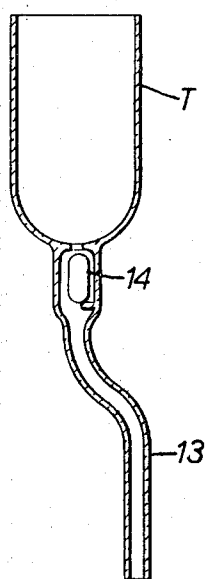


FIG. 3.

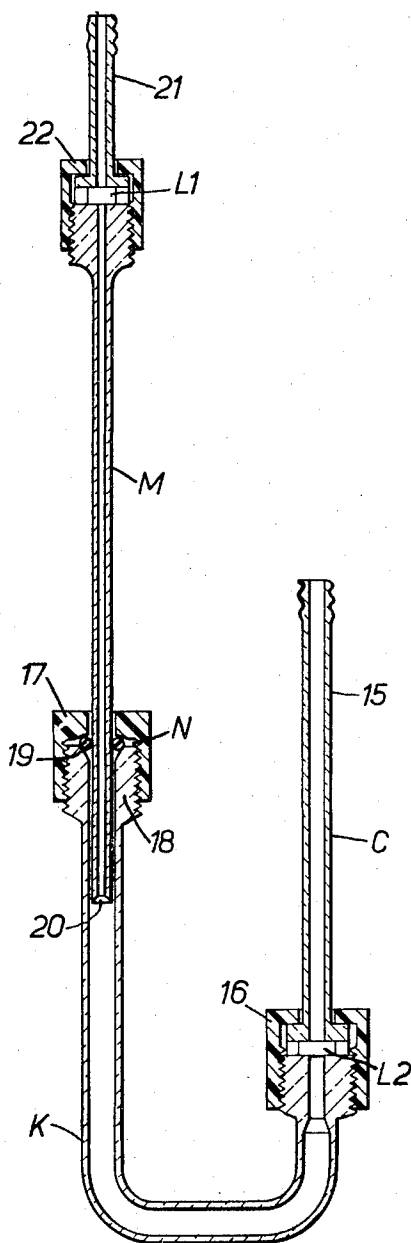


FIG. 4.

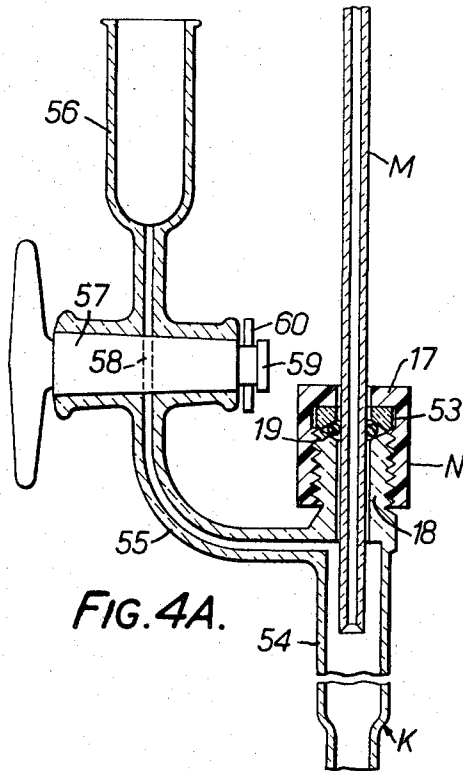


FIG. 4A.

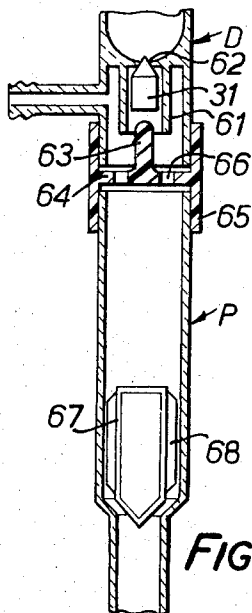


FIG. 5B.

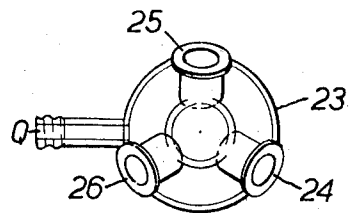


FIG. 5A.

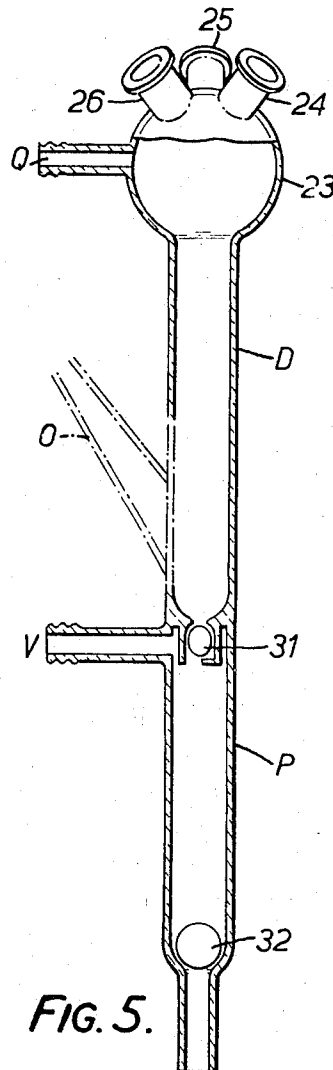
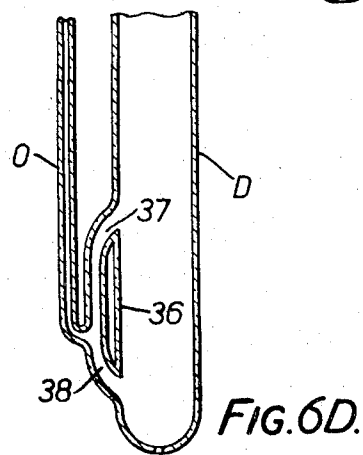
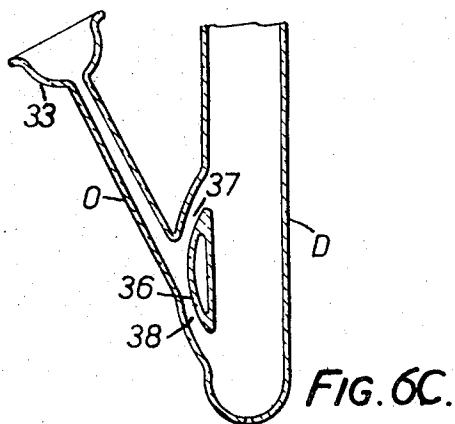
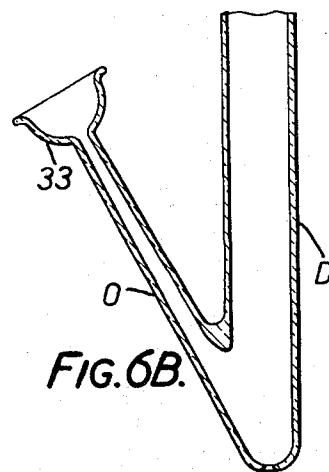
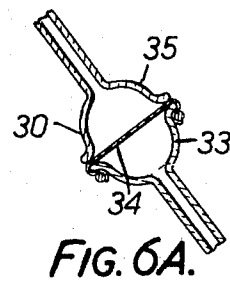
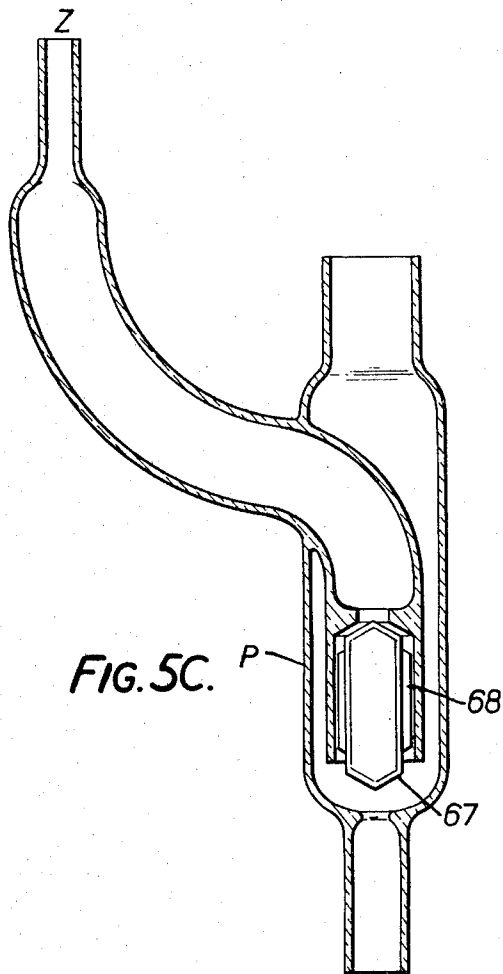


FIG. 5.



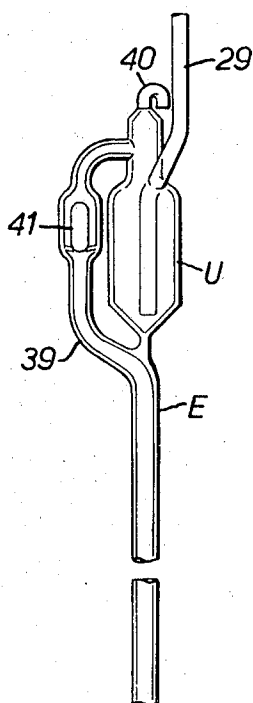


FIG. 7.

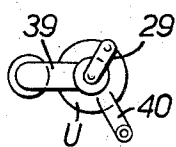


FIG. 7A.

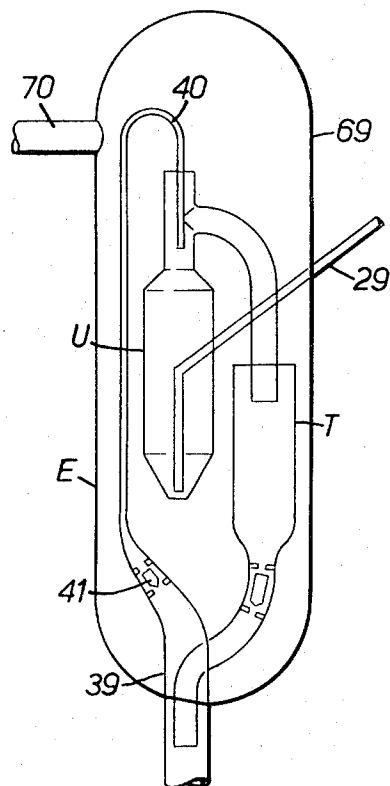
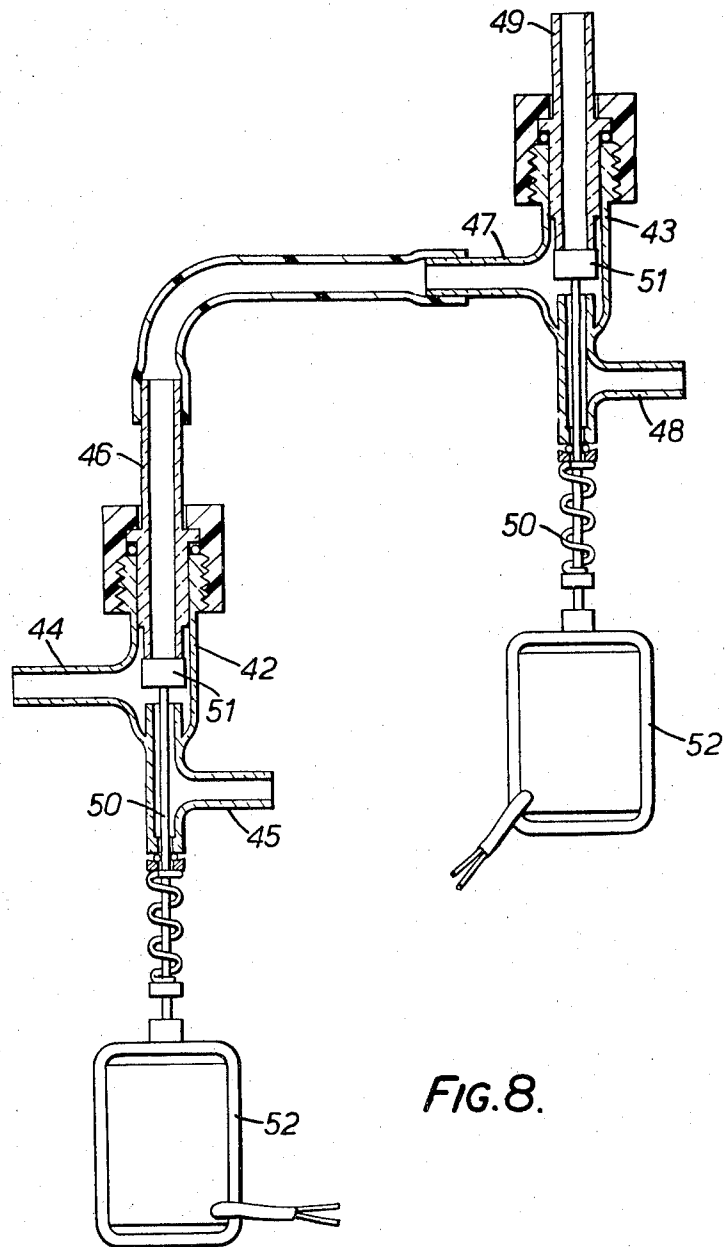


FIG. 7B.



AUTO-ANALYTICAL APPARATUS AND ANALYTICAL METHODS

This invention concerns improvements in or relating to an auto-analytical machine and method for performing chemical analyses of samples, such as body fluid samples in particular.

There are two basic situations in which the chemical analysis of body fluid samples are required. The first is where it is desired to obtain a fairly immediate indication of the chemical condition of a sample from a single patient under treatment (such as the determination of calcium in serum) so that the further treatment of the patient can be determined. This can be done, for example, in a test tube by a person used to handling such laboratory equipment as syringes, pipettes and titration assemblies but suffers from the disadvantages that it may take some time, that the laboratory technician may be absent or overworked and, most importantly, that the accuracy of the results may not necessarily be reliable and that the percentage error may be high where only a small sample is supplied.

The other basic situation concerns the analysis of a batch of samples of a similar character and this generally is performed on a continuous basis employing automatic apparatus. One such apparatus in general use employs a continuous flow system wherein reagents are pumped along a continuous pipeline and samples for analysis are injected intermittently into the stream, the integrity of the reagent sample mixtures being maintained by air bubbles in the stream. There may be twenty or more samples undergoing analysis travelling one behind the other in the stream at any one time. The sample/reagent mixtures are moved and measured by a peristaltic pump so that they pass through a dialyser, incubator, colorimeter (or other analysis device), and any other desired equipment.

Another batch treatment apparatus in common use is a discrete system apparatus wherein the samples are disposed in suitable cups loaded onto a turntable, which indexes round. Aliquots of each sample are withdrawn and transferred to test tubes on another turntable which indexes round in synchronism with the first turntable. Reagents are added at various stages and the results of the reactions are analysed, usually automatically, such as by a colorimeter. The samples and reagents are dispensed by means of syringes in determined amounts.

Both these types of automatic equipment are only really applicable for the handling of batches of samples and the samples spend 10 minutes or more in the machine, the reactions generally being run more or less to completion. Thus it is not only uneconomic to run the machine for the analysis of one sample only, but also the operating time is such that the analysis of a single sample could probably be effected more quickly by hand. The automatic equipment discussed above also suffers from the disadvantage that it involves a considerable cost outlay and is therefore only suitable for use in large analytical laboratories where it will be in substantially continual use.

It is an object of this invention to provide an auto-analytical machine of comparatively low cost which can be used for the analysis of single samples in a relatively short time by relatively unskilled persons. Such an apparatus could thus be used by a nurse in a hospital

to provide a rapid indication of a patient's condition determined from the analysis of a body fluid sample.

Accordingly, this invention provides, in one aspect, an auto-analytical machine for the analysis of liquid samples and comprising in combination a reaction chamber, a sample reservoir for holding the sample to be analysed, connecting tubing having one end directed into said reaction chamber and the other end being arranged to be able to dip into a sample in said sample reservoir, a pressure controlled sample measuring device adapted to move a predetermined quantity of sample into said connecting tubing, and a pressure control mechanism arranged to move said predetermined quantity of sample into said reaction chamber, said measuring device and said control mechanism being adapted to move said sample by a change of fluid pressure in said connecting tubing.

Whilst a reagent may be supplied to the reaction chamber, for an analytical chemical reaction with the predetermined quantity of sample by any convenient means, it is desired that it will in fact be supplied automatically to the reaction chamber, in a measured dose, upon operation of said pressure control mechanism. This is achieved by means of a liquid measurement unit, having a chamber, arranged to hold a measured quantity of reagent provided from a reservoir, connected by additional connecting tubing, to the reaction chamber.

A liquid measurement unit, suitable for the supply of reagent for use in the auto-analytical machine according to this invention, comprises a stand pipe situated within a liquid reservoir and opening at its other end into a chamber, a chamber outlet pipe, opening into an excess liquid receiver which is arranged to pass the excess liquid back into the reservoir, an inlet to said reservoir whereby pressure may be applied to force liquid through said stand pipe and into said chamber, and connecting tubing leading from the bottom of said chamber whereby the measured quantity of liquid in said chamber may be drawn off. If the outlet pipe leaves the chamber below the level at which the stand pipe opens into the chamber and does not rise above that level, then the volume of the measured quantity of liquid will be determined by the highest point of the outlet pipe. It is preferred however that the outlet pipe should rise above the level of opening of the stand pipe so that the volume of the measured quantity of liquid will be determined by that level. The provision of a non-return valve in the stand pipe may be desirable in certain cases.

Such a liquid measurement unit may be used also to supply diluent to the predetermined quantity of sample for analysis. In this case the reservoir will be a diluent reservoir and the end of the connecting tubing of the auto-analytical machine, into which the sample will have been drawn by the sample measuring device, will be the connecting tubing whereby the diluent will be withdrawn into the reaction chamber (together with the sample) upon operation of the pressure control mechanism. Ideally the chamber of the liquid measurement unit, and/or the connecting tubing will be mounted on a parallelogram of levers, or like apparatus, whereby the end of the connecting tubing may be applied to the sample reservoir and then be precisely repositioned in the chamber of the diluent measurement unit.

If the liquid measurement unit is for the supply of reagent to the reaction chamber, there may be occasions wherein it is desired to supply a particular reagent a short time after an initial reaction has commenced within the reaction chamber. In this case the reagent can be held within the chamber of the liquid measurement unit by enclosing the parts of the unit, apart from the reservoir and connecting tubing, within an envelope to which a negative or positive pressure may be applied respectively to hold the reagent within the chamber and subsequently inject the reagent, through the connecting tubing, into the reaction chamber.

A pressure controlled sample measuring device, suitable for use in the auto-analytical machine, ideally comprises a U-tube having a sintered disc at one end and filled with mercury from that end to a predetermined level on the other side of the U-tube, a second sintered disc at the other end of the U-tube, means for controlling the pressure on the side of said second sintered disc away from the U-tube and a column on the side of said first sintered disc away from the U-tube filled with a liquid which is immiscible with and of greater density than water.

If the column of immiscible liquid is connected to the connecting tubing situated between the sample reservoir and reaction chamber of the auto-analytical machine of this invention and the pressure at the other end of the U-tube is reduced by the pressure controlling means, the mercury will rise to fill the empty space (of known volume) within the U-tube, until it reaches the second sintered disc, thus drawing immiscible liquid through the first sintered disc into the U-tube, and drawing the known volume of sample into the connecting tubing. When the sample is subsequently drawn into the reaction chamber the pressure in the U-tube can be returned to normal at the same time.

The sintered discs used in the sample measuring device are preferably No. 4 glass sintered discs of about 1.5-mm. thickness, each fused into a solid glass annulus. The immiscible liquid used is ideally an organic liquid such as carbon tetrachloride.

Preferably, the other end of the U-tube will be made adjustable so that the volume of sample can be altered as desired. This is best done by forming the other end of the U-tube with a sealing gland having a movable capillary tube passing therethrough, with the second sintered disc at the other end of the capillary tube. The position of the capillary tube relative to the sealing gland will then determine the amount of space which the mercury can fill and thus the volume of sample which will be drawn in. The capillary tube may be calibrated to indicate the volume of sample which will be drawn in.

The filling of the U-tube, where a capillary tube is present, can cause difficulties in excluding all unwanted air. It is preferred in this case to provide a filling tube leading to the position directly below the sealing gland and provided with a stopcock, whereby mercury may be introduced into the U-tube and unwanted air bled off.

The reaction chamber itself will have openings in which the connecting tubing for the sample and reagent(s) may be secured. Ideally the connecting tubing will incorporate valves, which may be duckbill valves at the ends of the tubing where they enter the reaction chamber. It is preferred to provide some form of stirring arrangement for the liquids in the reaction cham-

ber so as to ensure a uniform reaction. This is best achieved by means of a side arm entering near the base of the reaction chamber whereby an oscillating pressure may be applied to the liquid in the lower portion of the reaction chamber. Ideally the side arm will terminate in a thistle funnel covered with a diaphragm and the oscillating pressure can then be applied to a further thistle funnel in contact with the diaphragm. It is preferred that an obstruction be positioned where the side arm meets the reaction chamber, so as to form two channels to give a more positive stirring action. At the bottom of the reaction chamber, an upwardly closing valve is desirable whereby the reaction chamber may be emptied. If the valve leads into a lower, emptying chamber having a downwardly closing lower outlet valve, the upwardly closing valve can be maintained closed by positive pressure applied to the emptying chamber. The downwardly closing valve is ideally less dense than water so that it will float whilst the emptying chamber is being emptied.

One or more reaction-determining transducers will be situated in or outside the reaction chamber. The transducer can be in the form of an electrode for measurement of pH, specific ion or the like or can be a colorimetric measurement device which will determine, for instance, whether the reaction has passed an end point which produces a colour change. Other transducers, applicable to automatic measurement may also be employed as required.

It may be desired to perform a titration operation within the reaction chamber and for this purpose a titration apparatus may be employed which will have an outlet tube leading into the reaction chamber. A suitable titration apparatus for this purpose would be a screw driven syringe, the position of the piston in the syringe being indicated by an appropriate linear transducer so as to indicate the amount of reagent which has been added until a suitable end point is reached as determined from the conditions in the reaction chamber. If a titration apparatus is used, then a releasable valve (such as an electro-magnetically controlled valve) should be present in the outlet tube leading to the reaction chamber so that the titration reagent will be prevented from entering the reaction chamber whilst negative pressure is being applied thereto in order to draw in the sample and any other reagent(s).

It will be appreciated that the auto-analytical machine, and the various parts thereof, as defined above, are operated mainly by the application of positive or negative pressure or atmospheric pressure at predetermined stages throughout the operation of the machine. These pressures may be applied by means of any convenient apparatus such as solenoid operated air valves connected to pressure pumps and vacuum pumps. The valve timing can be achieved by means of a cam timer or electrical circuitry.

It will readily be apparent that this apparatus is adapted for the analysis of single samples and employs a small amount of equipment as compared with batch treatment apparatus, and is therefore much less expensive to produce. Furthermore, the machine can be made to be virtually completely automatic, as controlled by the timing arrangement, so that any unskilled person can supply a sample to the machine and be provided with the analytical results of reactions occurring in the reaction chamber in the form of a written print out produced from the results supplied by the transduc-

ers monitoring the reactions. Furthermore, it is possible for the machine to be made so that the cycle of operation can be carried out in about 30 to 60 seconds with no prior preparation once the apparatus has been set up. This compares very favourably with the batch treatment machines which require a considerable time before they can be started up (as much as 30 minutes) and the time involved in carrying out the test can be as much as 10 minutes or more after the machine has started operating.

Although the auto-analytical machine of this invention has been described in relation to the analysis of samples of body fluids, it will readily be appreciated that this apparatus could be used for various other automatic operations such as dosages or analyses in mineral or organical chemistry, biochemistry, radiochemistry; or similar operations such as in pharmaceutical preparation, perfume preparations, distribution of microbial suspensions, distributions of serologic reactions, distributions of red blood corpuscles, serum and reagents to determine blood groups, distributions of radioactive solutions, preparations of radio-isotope dilutions, determinations of physical and physio-chemical characteristics: volume, weight, conductivity, pH, cryoscopic point and spectral absorption.

In order that the invention may be more fully understood preferred embodiments thereof will now be described, by way of illustration only, with reference to the accompanying drawings, in which:

FIG. 1 is a general schematic drawing of a system for chemical analysis of a sample;

FIGS. 2 and 3 show parts of a supply arrangement of diluent for the sample, in more detail than is shown in FIG. 1;

FIG. 4 shows, in more detail, the sample aspirating device of FIG. 1;

FIG. 4A shows a modification of a portion of the apparatus shown in FIG. 4;

FIGS. 5 and 5A show, in more detail, the reaction vessel of FIG. 1;

FIGS. 5B and 5C show modifications of the valve arrangements for the reaction vessel of FIG. 5;

FIGS. 6A, 6B, 6C and 6D show various stirring arrangements for the reaction vessel of FIG. 5;

FIGS. 7 and 7A show, in more detail, the reagent dispenser of FIG. 1;

FIG. 7B shows, diagrammatically, a modified form of the reagent dispenser shown in FIGS. 7 and 7A; and

FIG. 8 shows a control device for controlling the operation of the system shown in FIG. 1.

The system shown in FIG. 1 is designed to carry out quickly single chemical analyses on body fluids. It is intended for use in an emergency situation, and there will be a number of similar systems, each arranged to perform one specific test.

Basically the system is arranged to aspirate a carefully measured volume of serum or other liquid, wash this into a reaction vessel with a measured volume of diluent, add a measured volume of reagent(s) to the reaction vessel, stir the mixture, and observe the reaction with one of a number of transducers, for example, pH, specific ion, or coulometric electrodes, or colorimetric or fluorimetric transducers. The signals from these transducers will be handled electronically so as to provide a digital readout or printout.

The control of movement of liquids in this system is by what may be termed ternary fluidics, that is to say,

it is effected by opening portions of the system to atmospheric pressure or to pressures below or above atmospheric.

FIG. 1 shows the general arrangement of the system. In this drawing, a probe A to which the sample will be offered is shown disposed within a moveable vessel B mounted on a parallelogram of levers so that it can be removed and replaced in exactly the same position. The vessel B can be filled with diluent to the level I by temporarily pressurising vessel G. When vessel G is opened again to atmosphere, excess diluent will drain back into the vessel G leaving vessel B filled to level I. The funnel T which includes a non-return valve receives any overflow.

Vessel B and funnel T are shown in more detail in FIGS. 2 and 3 of the drawings. Vessel B has a chamber 10 of 8-mm. internal diameter and 20-mm. depth, with a neck 11 of 5-mm. internal diameter. The inlet tube 12 is of 1½-mm. bore and opens, at level I, 28 mm. above the bottom of chamber 10. Funnel T has an outlet pipe 13 of 5-mm. bore about 2.5 cms. long. The non-return valve 14 closes upwardly.

A device C, for aspirating the measured volume of sample from vessel B, is shown in greater detail in FIG. 4 of the drawings. It consists of a U-tube K, of approximately 5 to 6 mm. bore, the right-hand limb of which terminates in a sintered glass disc L2. The left-hand limb terminates in a gland N through which passes a capillary tube M which also terminates, at its upper end, in a sintered glass disc L1. This capillary tube M is adjustable in height through the gland N. The U-tube K is completely filled with mercury up to the level of disc L2 and also to the lower end of the capillary M. The length of connecting tubing 15 above disc L2 is filled with an organic liquid such as carbon tetrachloride which is immiscible with and of greater density than water. The sintered discs L1 and L2 (which are both No. 4 sintered discs, of about 1.5-mm. thickness, with a solid rim ground flat) are each fused into a solid glass annulus and are of a porosity grade such that they will permit the free passage of an organic solvent such as carbon tetrachloride but will exclude mercury at the pressures used in this system.

The connecting tubing 15 is of 3 mm. bore and is connected to the U-tube K, with the disc L2 therebetween, by a nylon union nut 16. The U-tube K has a bore no more than 3 mm. at the point where it meets disc L2. A nylon union nut 17 is also provided at the other end of U-tube K, whose threaded body 18 is chamfered so as to house, partly, the O-ring 19, which grips the capillary tube M and forms the gland N. The capillary tube M, which is of 1.5-mm. bore (preferably a precision bore), throughout its length, has a concave tip 20 at its lower end, and is connected, at its upper end, to connecting tube 21, with the disc L1 therebetween, by a nylon union nut 22. The capillary tube M is an easy sliding fit in the limb of the U-tube K and is of 12.0 cms. in length from tip 20 to disc L1.

FIG. 4A shows, in cross-section, a modification of the U-tube K and gland N which enables the mercury to be inserted in the U-tube with relative ease. The sealing of gland N is, incidentally, improved by the presence of the chamfered washer 53 which is desirably formed from PTFE. It will be noted that U-tube K is widened, at portion 54 which leads to gland N, so as to leave a free space of at least 1 mm. around the capillary tube M, to the depth to which it may be lowered, and that

a tube 55 leads off from the top of portion 54 (so as to leave a minimum of dead space) to a filling tube 56 via a stopcock 57 (shown not in cross-section) having a bore 58 and provided with a captive key 59 and spring retaining clip 60. Without the filling tube 56 and stopcock 57 arrangement, it is very difficult to remove all the air from U-tube K. However with the arrangement shown, air present in the top of enlarged portion 54 can escape through the carefully positioned filling tube 55. When the U-tube K and filling tube 55 are seen to be free from air, the stopcock 57 can be closed for normal operation of the whole apparatus.

When the pressure in the connecting tube 21 above disc L1 is reduced, mercury is drawn into capillary tube M until it reaches disc L1; carbon tetrachloride is drawn through disc L2 and the volume of liquid in the tubing is therefore increased; any sample offered to probe A by vessel B would therefore be drawn into the system to a measured volume.

The reaction vessel D of FIG. 1 is shown in greater detail in FIGS. 5 and 5A. The head 23 of vessel D has 3 apertures 24, 25, 26 set at about 120° to each other whereby small diameter tubes 27, 28 and 29 enter the vessel D (which has a bore of 10 mm. and a length of 8 cm. from head 23) from various units of the system. Tubes 27 and 29 terminate in non-return valves J which may be "duckbill" valves formed from a suitable chemically resistant elastomer. The tubes 27, 28 and 29 are ideally formed from PTFE or polythene with a good surface finish for good flow characteristics. A non-ionic surface active agent may be employed in the tubing to increase the ease of flow of the liquids used herein. The side arm O of reaction vessel D has a diaphragm arrangement 30 at its upper end which provides a system of stirring by oscillating the fluid (which will be of sufficient volume to cover the opening of side arm O into vessel D). Alternative arrangements to this are shown in FIGS. 6A, 6B, 6C and 6D, and will be described later. If the pressure at Q is reduced, liquid may be drawn into the reaction vessel from the various units of the system.

The lower chamber P of 7 cms. length is provided so that vessel D may be emptied via valve member 31 (formed from PTFE, polypropylene or polythene), without the effluent contaminating the vacuum control system. The lower valve 32 of this chamber is formed, as a ball, from polypropylene or polythene so that it is less dense than water (it may be hollow) and will float until chamber P is empty. A preferred valve arrangement is shown in FIG. 5B. The valve member 31 is shown as a cone-headed member in a cylindrical portion 61. Movement of the cone-headed member 31 is limited by the opening 62, at the base of vessel D, which is ground to as sharp an edge as possible so as to bite into member 31 and ensure a tight fit, and a boss 63. This boss 63 is set on a disc 64, integral with a sleeve 65 (formed from PVC), and provided with four drain holes 66. The sleeve 65 joins the part forming chamber P to the base of vessel D. The lower valve is provided with a hollow, closed, cylindrical valve member 67, adapted to seat within the outlet of chamber P and provided with three locating fins 68. Valve member 67 is formed from glass and is hollow so that it has a specific gravity less than 1.0. The modified valve seating shown in FIG. 5C enables the valve member 67 to be lifted and held up by applying reduced pressure at V while the chamber P drains.

FIGS. 6A to 6D show various alternative experimental stirring arrangements for the side arm O of vessel D. The diaphragm arrangement 30 shown in FIG. 1 is detailed in FIG. 6A and is also used in the arrangements of FIGS. 6B and 6C. Arrangement 30 comprises thistle funnel 33, on arm O, over the mouth of which is stretched a rubber diaphragm 34. Another thistle funnel 35 mates with the diaphragm 34 with a rubber gasket. The rims of funnels 33, 35 should be flat but need not be ground.

In the arrangement of FIG. 6B the arm O is shown to have a narrow bore which widens slightly from funnel 33 towards vessel D. In FIG. 6C (which is the presently preferred arrangement) a similar arrangement to that of FIG. 6B is shown wherein the bore of arm O near funnel 33 is about 2 mm., widening to about 3 mm., where it opens into vessel D, at which point an obstruction 36, forming upper and lower passages 37 and 38, is positioned. In FIG. 6D no funnels or diaphragm are used and an arm O of 1-mm. bore leads to an obstruction 36 forming passages 37 and 38 of 3mm. bore which open about 20 mm. apart from each other. The stirring arrangements will be fed with compressed gas so as to effect stirring.

The unit on the extreme left hand side of FIG. 1 and designated E is the reagent dispenser which is shown in more detail in FIGS. 7 and 7A. In vessel H is pressurised, liquid is forced via pipe 39 (which is about 15 cm. long and of 5-mm. bore), up into the upper chamber U until it overflows from the capillary tube 40 into a funnel T (FIG. 1) similar to that shown in FIG. 3. A downwardly seated non-return valve 41 in the stand pipe 39 prevents liquid from running back into vessel H. The chamber U is about 25 mm. long with a 10-mm. bore at its widest part and has a volume of approximately 2 ml. The tube 29, leading to vessel D, enters chamber U, to the bottom thereof, as a thin walled capillary tube of about 1.0-mm. bore. The three limbs 29, 39 and 40 of vessel U are sited at about 120° to each other, as seen in FIG. 7A. Differing reagent dispensers having a chamber U of approximately 1 ml. or 3 ml. (or more) may be required depending upon the reaction requirements and it will be appreciated that the volume of chamber U does not need to be known exactly as it is only necessary that the amount of reagent supplied should be the same for the test reaction(s) as it is for the standard reactions.

The reagent dispenser shown in FIG. 7B has some minor constructional differences to that shown in FIG. 7, but also has an enclosing, sealed envelope 69 to which may be applied variable pressures via pipe 70. With such an arrangement, the reagent can be supplied at a predetermined time after a reaction has commenced within vessel D. This is done by applying reduced pressure to pipe 70 at the same time as it is applied at Q so that the reagent is restrained from leaving chamber U. Then at the required time positive pressure can be applied via pipe 70 to inject the reagent into vessel D. If one or more such dispensers are used, they will be additional to the dispenser for supplying an initial reagent, and thus head 23 of vessel D may have to be formed to accommodate extra inlet tubes.

Unit F, which may optionally be incorporated in the system, is a means of supplying a varied and measurable volume of liquid as for titration and is in the form of a screw driven syringe 71. The position of its piston 72 (driven from unit 73) will be indicated by an appro-

appropriate linear transducer. The non-return valves 74 provide a means of refilling the syringe 71 automatically when piston 72 returns. Electro-magnetic 75 enables the upper valve 76 to be held shut when pressure is reduced in vessel D so that the syringe system is closed off.

The operation of the system is as follows, assuming the system to be primed, so that the tubing 27 to the right of the reaction vessel D will be filled with diluent (normally distilled water together with a non-ionic surface active agent):

1. Positive pressure is applied to V closing both valves 31, 32 in chamber P.

2. Vessel B is lowered to expose the probe A.

3. The sample is offered to probe A and reduced pressure is applied to W. A measured volume of sample is thus drawn into the probe.

4. Vessel G is pressurised for a short time to ensure that vessel B is filled to the level I and then vessel B is replaced to enclose probe A.

5. Vessel H is pressurised for a short time so that chamber U is filled with reagent.

6. Reduced pressure is applied to Q so that the contents of chamber U, the sample in probe A, and the contents of vessel B are drawn into the reaction chamber D until all the tubing 27, 29 is emptied. During this period W is opened to atmosphere so that the mercury in U-tube K may fall back to its normal "stand-by" position, taking about 3 seconds to fall, although a one second delay may be provided if necessary.

7. Q is opened to atmosphere.

8. A pulsatile pressure is applied to X thus oscillating the fluid. Appropriate optical or other physical observations are made on the liquid.

9. After an adequate period of observation the positive pressure on V is reversed and the reaction mixture drawn into chamber P. A short positive pulse serves to empty P to waste.

10. In order to initiate cleaning of the system, as a first step continuous pressure is applied to vessel G so that vessel B remains filled with distilled water.

11. Vacuum is applied for a short time to Q so that distilled water is drawn into the reaction chamber D.

12. Vacuum is applied to V to empty vessel D.

13. Steps 11 and 12 are repeated twice.

14. Finally, vessel G is brought to atmospheric pressure leaving the right hand side of the system still filled with distilled water.

Control of the connection of each of the various units to compressed air, atmosphere, or vacuum may be achieved by means of a pair of three way valves 42, 43 arranged as shown in FIG. 8.

Valve 42 has its main junction 44 connected to one of the instruments, and its secondary junctions 45, 46 respectively connected to vacuum and the main junction 47 of the other valve 43. The secondary junctions 48, 49 of the other valve 43 are respectively connected to atmosphere and a source of compressed air. Each valve 42, 43 is operated by a solenoid-controlled, spring-biased plunger 50 whose head 51 can selectively close one or other of the secondary junctions. Thus the instrument can be connected to vacuum, atmosphere or the compressed air source by suitable selection of the states of valves 42, 43. The body of each valve is to be made of glass or suitable plastic, the moving parts of nylon and, in order to minimise power dissipation, the solenoids 52 will be of the "self-hold" type.

Control of the sequence of operations may be by such means as a cam timer or an oscillator-controlled solid state logic circuit. In order to achieve the short analytical time required of this system, much use will have to be made of reaction rate methods. In most cases these will be non-linear, and appropriate corrections will be made electronically.

Examples of use of the apparatus shown in the accompanying drawings will now be described to show how various titration reactions may be carried out to measure the amount of certain chemicals present in liquid samples.

Example 1 — Calcium Measurement.

This is based on the complexometric titration of calcium with ethylene diamine tetra acetic acid (EDTA) using the fluorescent indicator "calcein" (fluorescein complexone) to indicate the end point.

The sampler is set to draw up 0.1 ml of sample in probe A and a reagent dispenser E is chosen which will dispense 3.0 ml. The vessel H of dispenser E is charged with 0.4 M. potassium hydroxide containing 4.0 mg./litre of calcein. The vessel of the titrating syringe F is charged with EDTA (Disodium salt) 0.93 g./litre.

A standard solution containing 100 mg./litre of calcium in the form of the chloride is used to set the machine.

The cycle of operations is as follows: 0.1 ml of standard solution is drawn into the probe A and vessel U is filled with 3.0 ml of the potassium hydroxide containing calcein. Both are drawn into the cuvette D and the standard solution is followed by 1.0 ml of distilled water from vessel B to ensure that it is completely carried into D. The cuvette is illuminated by a beam of light (Max 435 nm) and the fluorescence is observed at right angles to the light by a photocell.

The syringe F is set in operation, discharging EDTA into the cuvette and this continues until the green fluorescence disappears whereupon the photocell triggers an electronic switch which stops syringe F. The stroke of the piston is measured by a linear transducer and the signal is stored in a digital register. The wash cycle is carried out and the process repeated, using 0.1 ml of the serum to be measured. The signal representing the stroke of the piston for this measurement is stored in a second register. An arithmetic unit divides the second result by the first and multiplies it by the factor 10 to give the answer to a readout device which reads in mgm/100 ml.

Example 2 — Total Protein Measurement.

This is measured utilising the biuret reaction which is specific for peptides, polypeptides and proteins. These react with alkaline copper tartarate to give a violet coloured solution which has a maximum absorbance at 560 nm. The absorbance of the coloured compound is directly proportional to the concentration. The reaction is 80% complete in one minute at room temperature.

BIURET REAGENT.

1. Alkaline Iodide — 16.0 g. Sodium hydroxide and 10.0 g. Potassium iodide in 1 litre water.

2. Stock Biuret reagent — 16.0 g. sodium hydroxide, 90 g. sodium, potassium, tartarate, 30 g. copper sulphate (5 H₂O) and 10.0 g. potassium iodide, made up to 1 litre with distilled water.

3. Working Biuret reagent — Dilute 200 ml stock biuret to one litre with alkaline iodide solution.

Human or bovine albumen solutions containing 5.0 g/100 ml is used as a standard. The sampler is set to draw up 0.1 ml in probe A and the vessel U of a 3.0 ml reagent dispenser E is charged with working biuret.

0.1 ml of standard solution is drawn into the probe A and hence into the cuvette D followed by 1.0 ml of distilled water from vessel B. At the same time 3.0 ml of working biuret reagent is drawn into the cuvette D from vessel U. A beam of light 560 nm is passed through the cuvette and the emerging light is measured by a photocell. The logarithms to the base 10 of the signals from the photocell are observed, and the difference between the initial signal and the signal after 60 seconds is stored in a register as before.

The process is repeated using the unknown serum and the result stored in a second register. The arithmetic unit divides the second result by the first and multiplies the result by 5 to give the answer in g./100 ml. Example 3 — Lactate Dehydrogenase (DHL) Measurement.

This enzyme converts pyruvate to lactate. The hydrogen donor for the reaction is reduced nicotinamide adenine dinucleotide (NADH). In donating its hydrogen the latter is converted to the oxydised form (NAD) which absorbs in the ultra violet at 340 nm. The rate of the reaction is directly proportional to the concentration of LDH, and is measured by observing the rate of change of absorbance at 340 nm.

REAGENTS.

1. Substrate — Phosphate Buffer 50 mM, pH 7.5 containing 0.3 mM sodium pyruvate.

2. NADH — 0.5 mM, NADH in water.

The substrate is dispensed from a 3.0 ml dispenser E and the NADH from another, 1.0 ml. dispenser E.

0.1 ml of the serum under test is drawn into cuvette D and rinsed in with 1.0 ml of distilled water. At the same time 3.0 ml of phosphate buffer and 1.0 ml of NADH are drawn into the cuvette from dispensers E. An ultraviolet beam at 340 nm is directed through the cuvette and its intensity is measured by a photocell. The logarithm of the voltage output is observed and the gain of the system is so arranged that unity voltage output is equal to 1.0 Optical Density units. The change in voltage output over 30 seconds is recorded and placed in the register. The arithmetic unit multiplies this value by 10,128 to express the result in milli International units per ml. It should be noted that no standard is necessary in this test.

I claim:

1. Auto-analytical apparatus for the analysis of liquid samples and comprising in combination a reaction chamber, connecting tubing, one end of which is directed into the reaction chamber and the other end of which is open and free to dip into a supply of a sample to be analysed, a pressure controlled measuring device provided with fluid pressure changing means, the measuring device being connected between the two ends of the connecting tubing so as to be able to draw a predetermined quantity of a sample into the open other end of the connecting tubing, a pressure control mechanism which, when operated, will cause a change of fluid pressure in the reaction chamber and the connecting tubing serving to draw any liquid between the two ends of the connecting tubing into the reaction chamber, and reagent means for supplying reagent to the reaction chamber for reaction with any sample introduced thereinto.

2. Apparatus as claimed in claim 1 wherein the reagent means comprises a reagent reservoir, a reagent chamber for holding a measured quantity of reagent provided from the reservoir, supply tubing from the reagent chamber to the reaction chamber, supply means for delivering said measured quantity to the reaction chamber, and means for moving reagent from the reservoir to the reagent chamber.

3. Apparatus as claimed in claim 1 wherein the reagent means comprises a reagent reservoir, a reagent chamber for holding a measured quantity of reagent provided from the reservoir, supply tubing from the reagent chamber to the reaction chamber, and supply means for delivering said measured quantity to the reaction chamber, the reagent means also including a stand pipe having one end situated within the reservoir and opening at its other end into the reagent chamber, an overflow from the reagent chamber, an excess liquid receiver interconnected between the overflow and the reservoir, an inlet to the reservoir whereby pressure may be applied to force liquid through the stand pipe and into the reagent chamber, said supply tubing leading from the bottom of the reagent chamber whereby the measured quantity of liquid in the reagent chamber may be drawn off.

4. Apparatus as claimed in claim 3, wherein an envelope encloses parts of the reagent means apart from the reagent reservoir and supply tubing, such that a negative or positive pressure may be applied by pressure control means respectively to the envelope to hold the reagent within the reagent chamber and subsequently inject the reagent out through the supply tubing.

5. Apparatus as claimed in claim 1 including a diluent reservoir, and a diluent chamber connected to be supplied from the diluent reservoir, the diluent chamber being provided adjacent the other end of said connecting tubing of the auto-analytical machine, into which the sample is to be drawn by the sample measuring device, said chamber being movable relative to the connecting tubing whereby diluent may be withdrawn into the reaction chamber together with the sample upon operation of the pressure control mechanism.

6. Apparatus as claimed in claim 5 including a stand pipe having one end situated within the reservoir and opening at its other end into the diluent chamber, an overflow from the diluent chamber, an excess liquid receiver interconnected between the overflow and the reservoir, an inlet to the reservoir whereby pressure may be applied to force liquid through the stand pipe and into the diluent chamber, said chamber being movable relative to the connecting tubing whereby a measured quantity of liquid in the diluent chamber may be withdrawn.

7. Apparatus as claimed in claim 1 wherein the pressure controlled sample measuring device comprises a U-tube, a sintered disc at one end of the U-tube, mercury filling the U-tube from that end to a predetermined level on the other side of the U-tube, a second sintered disc at the other end of the U-tube, means for controlling the pressure on the side of said second sintered disc away from the U-tube and a column on the side of said first sintered disc away from the U-tube filled with a liquid which is immiscible with and of greater density than water, the column of immiscible liquid being interconnected with the connecting tubing.

8. Apparatus as claimed in claim 7 wherein adjustment means are provided to adjust the positioning of

the other end of the U-tube so that a predetermined quantity of sample can be altered as desired.

9. Apparatus as claimed in claim 7 wherein a sealing gland is formed at the other end of the U-tube, a capillary tube movable by adjustment means has one end passing through the sealing gland, and the second sintered disc is positioned at the other end of the capillary tube, such that movement of the adjustment means will cause said predetermined quantity of sample to be varied.

10. Apparatus as claimed in claim 7 wherein a filling tube leads to a position directly below the other end of the U-tube and a stopcock is provided in the filling tube, whereby mercury may be introduced into the U-tube and unwanted air bled off.

11. Apparatus as claimed in claim 1 incorporating a stirring arrangement for the liquids in the reaction chamber comprising a side arm entering near the base of the reaction chamber and pressure varying means associated with the side arm whereby an oscillating pressure may be applied to air in the side arm to cause movement of liquid in the lower portion of the reaction chamber.

12. Apparatus as claimed in claim 11, wherein a thistle funnel is formed at the termination of the side arm, a diaphragm covers the thistle funnel, and a further thistle funnel is provided in contact with the diaphragm so that an oscillating pressure can then be applied to the diaphragm.

13. Apparatus as claimed in claim 11, wherein an obstruction is positioned where the side arm meets the reaction chamber, and two channels are formed by that obstruction.

14. Apparatus as claimed in claim 1, wherein an upwardly closing valve is provided at the bottom of the reaction chamber.

15. Apparatus as claimed in claim 14, wherein a lower emptying chamber is provided below the valve and a downwardly closing lower outlet valve is provided at the outlet of the emptying chamber whereby the upwardly closing valve can be maintained closed by positive pressure applied to the emptying chamber.

16. Apparatus as claimed in claim 1 incorporating a titration apparatus having an outlet tube leading into the reaction chamber.

17. Apparatus as claimed in claim 16, wherein the titration apparatus is a piston-carrying screw-driven syringe, and incorporates a linear transducer, whereby the position of the piston in the syringe will be indicated by the linear transducer so as to indicate the amount of reagent which has been added until a suitable end point is reached as determined from the conditions in the reaction chamber, the syringe having a outlet tube leading to the reaction chamber.

18. Apparatus as claimed in claim 16, wherein a re-

leasable valve is present in the outlet tube leading from the syringe, so that the titration reagent may be prevented from entering the reaction chamber.

19. Apparatus as claimed in claim 1, wherein solenoid operated air valves, for connection to pressure pumps and vacuum pumps, are provided for the application of positive or negative pressure or atmospheric pressure at predetermined stages throughout the operation of the machine.

20. A method of analysis of a liquid sample comprising the steps of drawing a predetermined quantity of the sample into one end of connecting tubing by a change of fluid pressure under control of a pressure controlled sample measuring device interconnected to an intermediate portion of the connecting tubing, moving the predetermined quantity of the sample into a reaction chamber to which the other end of the connecting tubing is applied by a further change of fluid pressure in the reaction chamber and the connecting tubing under control of a pressure control mechanism, supplying reagent to the reaction chamber and carrying out an analytical reaction on the predetermined quantity of sample in the reaction chamber.

21. A method as claimed in claim 20, wherein a diluent is drawn into the reaction chamber, together with the sample, upon operation of the pressure control mechanism.

22. A method as claimed in claim 20, wherein a measured quantity of reagent is moved through tubing from a reagent chamber to the reaction chamber by a change of fluid pressure in the tubing under control of the pressure control mechanism.

23. A method as claimed in claim 22, wherein the predetermined quantity of sample and the measured quantity of reagent are moved into the reaction chamber at the same time.

24. A method as claimed in claim 20, wherein a titration reaction is carried out in the reaction chamber by adding a titration reagent from titration apparatus in the form of a piston-carrying screw-driven syringe, and incorporating a linear transducer, whereby the position of the piston in the syringe will be indicated by the linear transducer so as to indicate the amount of reagent which has been added, through an outlet tube leading to the reaction chamber, until a suitable end point is reached as determined from the conditions in the reaction chamber.

25. A method as claimed in claim 20, wherein the liquids within the reaction chamber are stirred during the progress of the reaction by means of an oscillating pressure applied to the liquid in the lower portion of the reaction chamber, through a side arm entering near the base of the reaction chamber.

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