sodium fluoride with 1.00 part sodium chloride was satisfactory in all respects. The mixture fused easily at about 900° C. in a platinum crucible cover over a Bunsen or Mekker burner and, on rapid cooling in contact with a cold metal block, formed a fine-grain disc, easily separable from the platinum and sufficiently durable to withstand ordinary handling. A disc weighing 2 grams and 30 mm. in diameter was found satisfactory. The crucible covers used were shaped on a hardwood die and cleaned prior to each use by repeated fusions with the flux. The fluorescence of uranium-containing discs was increased by heating at bright red heat for 7 minutes, but decreased on more prolonged heating. Interference by several of the substances listed in Table 1 was reduced by heating, maximum fluorescence being attained in most cases in from 7 to 10 minutes.

Under the conditions cited, the fluorescence of the discs, as measured by the galvanometer deflections, showed a nearly linear relation to the uranium content over the range 0.2–50.0 μg. uranium/gram flux. At higher concentrations of uranium, up to 300 μg./gram flux, a smooth curve was obtained. (The maximum reported by Nichols and Slattery (*3*) is found at much higher concentrations.) The curves were used directly as working curves, by standardizing the instrument with a uranium glass disc at frequent intervals and making the occasional slight correction required by the blank. This type of instrument is capable of very great sensitivity, although the accuracy might be considerably improved by substitution of a phototube balanced circuit for the single barrier-layer cell.

It was required that the second fluorophotometer be portable, light, and able to withstand rough handling. Since great accuracy was not essential, an exceedingly simple type of visual comparator was used. This consisted of a comparator wheel on which were mounted standard alkali fluoride discs and a holder for the disc to be examined. The concentration of uranium in the standard discs varied geometrically from 0.1 to 200 μg./gram flux. The source of ultraviolet was a General Electric 4-watt RP-12 lamp, which may be operated from a 24-volt battery through a variable 25-ohm resistance. This lamp radiates principally in the vicinity of 365 mμ and is far more effective than the common 110-volt argon glow lamp. A Corning Glass Works filter No. 584 was used to eliminate practically all visible light. The instrument, including batteries, was housed in a specially designed plywood case measuring 6 x 7 x 10 inches (Fig. 1). The weight was only 4.8 pounds. The method of preparing discs for use in the portable comparator is similar to that mentioned above, except that, in field use, a gasoline blowtorch is used, and necessary weighings are accomplished with a portable balance. Sampling is more time consuming than the actual analysis, which requires 20 minutes at the most.

Because of the requirements imposed, standard techniques for the chemical separation of uranium from samples were not considered feasible. Under ordinary conditions, the method of Hoffmann (*2*) is satisfactory but tedious. A semiquantitative study of the effect of relatively large amounts of impurities was undertaken and is recorded in Table 1. Of the substances studied, antimony, bismuth, cadmium, cerium, cobalt, chromium, iron, lead, manganese, and zinc were found to interfere seriously, because they quench the fluorescence due to uranium, obscure it by coloring the flux, or render the disc brittle. Northup (*4*), in a recent article, gives a more extended discussion of substances which interfere qualitatively with the fluorescence of sodium fluoride beads, together with a study of the effect of columbium, the one element which shows fluorescence similar to that due to uranium.

Several possible procedures for inactivating or volatilizing the above-mentioned impurities were tried. It might be expected that many of these elements could be volatilized as the halide by heating with ammonium chloride, hydrofluoric acid, etc. This procedure was fairly successful with iron, but in all cases significant amounts of uranium were lost. Attempts to volatilize chromium as chromyl chloride were unsuccessful for the same reason. Dilution of the sample with flux did not greatly alter the extent of interference, except that fragility was decreased.

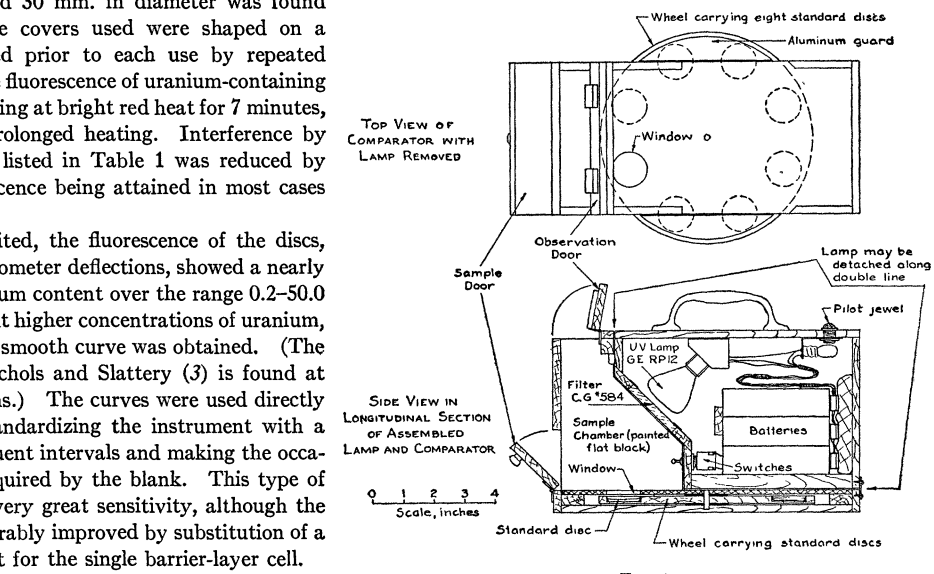

FIG. 1

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A Simple Resistance Thermometer for Blood-Temperature Measurements[1]

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In connection with an instrumentation program for the Medical Research Laboratory, Edgewood Arsenal, a compact field instrument has been developed for the intravenous meas-

[1] This work was carried out under contract with the Medical Division of the Chemical Warfare Service and was under the direction of A. H. Pfund. The authors wish to thank Capt. Lawrence Hobson (MC), AUS, for whom the instrument was constructed, for his suggestions, enthusiastic use of the instrument, and patience. We are indebted to Mr. C. B. Green, of the Bell Telephone Laboratories, for supplying the thermistor elements and pertinent information.

[2] Now with Leeds and Northrup Company, Philadelphia, Pennsylvania.

urement of animal blood temperature in the vicinity of the heart. The instrument, a resistance thermometer, is compact, easily constructed, fast responding (5 seconds), accurate, and rugged.

The Temperature-sensitive Element and Its Mounting

The temperature-sensitive element is a small, glass-coated, spheroidal thermistor bead (Western Electric V-642) with a resistance of about 1,300 ohms at 36° C. and a temperature coefficient of about −3.5 per cent at this temperature. For insertion into a vein through a 12-gauge hollow needle, the thermistor bead is mounted on the end of a 5-French catheter[3] as shown in Fig. 1.

The thermistor bead fits into a spherical recess in one end of a small copper cylinder, the other end of which is cut down

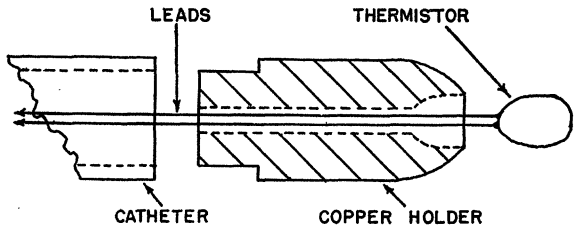

Fig. 1. Thermistor mounting details.

to match the inside diameter of the catheter. The cylinder is 0.070 inches in diameter, ¼ inch long, and has a hole (No. 72 drill) bored through its length for the thermistor leads.

Two-foot lengths of No. 32 gauge enameled copper wire are soldered to the thermistor leads, after which the soldered junctions and the thermistor leads are given a few insulating coats of thinned glyptal varnish. These leads are then threaded through the copper holder and the catheter. The copper holder is cemented into the end of the catheter with glyptal, and the bead is similarly cemented into the recess in the end of the copper. After a few hours this end of the catheter is dipped in thinned glyptal and dried in air. This is repeated until the whole assembly presents a continuous, smooth surface. Heavier copper wires are soldered to the No. 32 gauge leads at the other end of the catheter, the junctions varnished, and the wires and catheter tightly taped together. Only two leads are used because the relatively high resistance change of the element with temperature makes compensating leads unnecessary.

Associated Electrical Circuit

The circuit which translates thermistor resistance values into temperature is a conventional Wheatstone network with the thermistor in one arm. The bridge is balanced for a thermistor temperature of 46.0°, and other temperatures are read as unbalance. Since the indicating meter is a zero center meter, and the unbalance is linear for thermistor temperatures within a 10° C. range on either side of 46.0° C., the thermometer indicates temperature linearly from 36.0° C. to 56.0° C. The scale is marked off in 0.2° C. divisions and can easily be read to 0.1 ° C.

The complete electrical circuit is shown in Fig. 2. Two controls (*A*, *B*) are provided: *B* permits adjustment of the balancing resistance, and *A*, limited adjustment of the bridge potential. In order to avoid temperature errors due to heating of the element by the bridge current, a maximum of 3 volts is applied to the bridge. The bridge meter (*M*) is a Triplett Model 625 microammeter (1,000 ohms) set on the 100-0-100 microampere scale. A double throw switch allows the temperature-indicating meter to be used in standardizing the bridge voltage. The switch cuts the meter out of the bridge and puts it in series with a fixed resistance and the bridge potential. The switch has three positions (*S*, *R*, *T*). Position *S* uses the meter for bridge-voltage standardization.

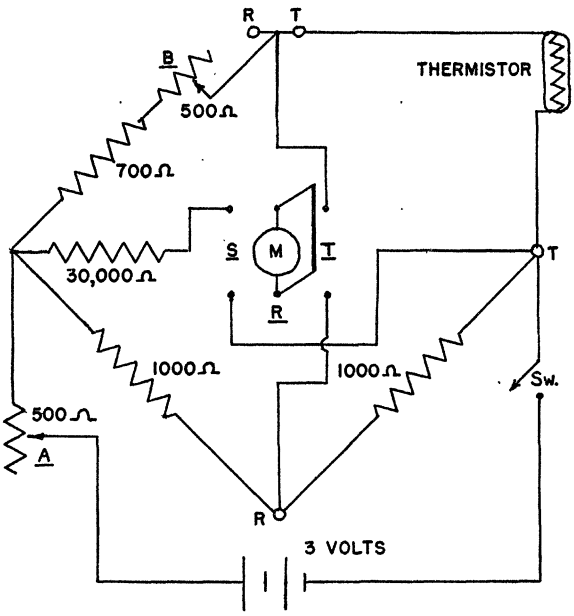

Fig. 2. Bridge circuit diagram.

With the switch in position *T*, *M* indicates the temperature of the thermistor. Position *R* disconnects the meter (*M*) from the circuit for use of auxiliary recording apparatus.

The most striking instrumental feature of the bridge is the location of all bridge elements, controls, switches, and batteries inside the case of the meter. This Triplett case measures 5½ x 6 x 2½ inches; hence, the complete bridge and meter forms a very compact unit. The zero-adjusting rheostat (*B*) and voltage-standardizing rheostat (*A*) are mounted on one side of the case, and the two switches on the other. The four terminals atop the Triplett case have been rewired as the two terminals marked T and the two marked R on the diagram. The T terminals provide bridge connections for the thermistor, and the R terminals are provided in case it is desired to use auxiliary recording equipment.

After preliminary calibration, the only check that needs to be made on the instrument before use at a later date is a voltage check, which consists of removing the thermistor, snapping the switch to position *S*, and adjusting *A* until the meter gives the standard deflection corresponding to the

[3] We have found an excellent source of very inexpensive catheters in Belden 8941 No. 20 solid lead wire with braid-lacquered insulation. The insulation is slipped off a two-foot length of wire, the loose white inside insulation is removed, and the remaining length of waterproof lacquered braid constitutes a catheter. The flexibility of the catheter is ideal for the present application.

correct bridge voltage. This deflection is determined by the preliminary calibration. After the voltage check, the thermistor is connected to the bridge, and the instrument is ready for use.

Conclusions

Several months' use of this instrument in field work under extreme conditions has proved its ruggedness and ease of handling. It has filled a definite need in the Edgewood work and should be quite useful wherever an accurate, simple, and fast thermometer is necessary in physiological studies. The introduction of another matched temperature-sensitive element in the opposite bridge arm would make this instrument applicable to the measurement of temperature differences.

The Cultivation of Mammalian Liver Cells in Large Numbers[1]

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The cultivation *in vitro* of cells of ectodermal origin may become important in certain investigations, particularly in the application of tissue culture to virus studies, biochemicar analyses, and studies of malignancy. However, the cultivation of such cells presents difficulties because of the greatel facility of growth of the connective tissue elements present in such explants.

The technic to be described was developed in the course of experiments on the attempted cultivation of the agent of infectious hepatitis. This method made it possible to produce cultures containing large numbers of liver cells with few fibroblasts, without employing a very elaborate technic. The method is based on the roller-tube technic of Gey (*2*), with the following modifications: the random distribution of many pieces of explanted tissue in roller tubes, and the use of a medium which selectively favors the growth of epithelial cells.

Materials

For a typical experiment involving 24 tubes the following sterile materials were required: 24 acid-free and grease-free test tubes, 18 x 150 mm.; a few capillary pipettes about 3 mm. in diameter at the tip; a few capillary pipettes, 200 mm. long, with the usual diameter at the tip (about 1 mm.) and bent at a 45° angle at a point 2–3 mm. from the tip; 24 solid rubber stoppers to fit the culture tubes; 3–50-cc. pointed centrifuge tubes; 1:600 heparin solution; 3–20-cc. bulb pipettes; a Petri plate, 100 mm. in diameter, containing a watch glass; a syringe and needle suitable for withdrawing 20 cc. of heart's blood from a rabbit; and a few dissecting instruments, including one fine, small, curved scissors, preferably strabismus scissors. A small amount of ice and rubber bulbs or tubing for manipulation of the pipettes are also needed.

Assembling the Culture

The explant material was obtained from a rabbit 7–10 days old, lightly anesthetized and exsanguinated. A portion of the liver was removed aseptically and placed in the watch glass contained in the Petri plate. The tissue was minced with strabismus scissors until the bits appeared to be about 1 mm. in diameter. The minced tissue was then suspended in Gey's solution (*2*) in the watch glass and transferred to a 50-cc. centrifuge tube. The top of the Petri plate was used as a shield to prevent contamination from the air. About 20 cc. of Gey's solution were then added to the tissue suspension in the tube. The latter was rotated until the tissue was distributed and was then allowed to stand. After a few minutes all the particles of about 1 mm. had settled below a well-defined plane, leaving a supernate containing erythrocytes and minute fragments. This supernate was removed by suction, and the washing of tissue fragments in Gey's solution was repeated twice more. After the supernate was removed for the third time a wide capillary pipette with rubber bulb was used to distribute small amounts of the sediment into the cotton-plugged culture tubes. The volume of the rather watery sediment which was picked up each time for placing in the culture tubes was such as to contain about 60 pieces of tissue in about 0.3 cc. of the saline solution.

The tissue was distributed by means of the capillary pipettes with bent tips. Throughout the procedure the culture tube was kept horizontal to avoid contamination from the air. The technic of distribution was as follows: The pipette was used to spread the small amount of fluid over the lower half of the tube until the entire surface was moistened, and the clump of fragments was distributed in a rough ring around the tube. Then the point of the bent tip was turned toward the surface of the tube and run up and down between the bottom of the tube and approximately halfway to the open end, while the left hand slowly rotated the tube. Bits of tissue were thus distributed over the entire lower half of the tube. Clumps remaining after this procedure were pushed apart by the tip of the capillary pipette, and any noted gross unevenness of distribution was corrected. Quite often many pairs of tissue fragments were left in contact by this procedure, but since the purpose of the method was to obtain a maximum total circumference of explants in as little time of manipulation as possible, this was not regarded as objectionable. Thus, 60 fragments which included 10 contiguous pairs would reproduce the effect of 50 entirely separate pieces. After the tissue fragments had been placed in all the tubes they were fixed in position by adding to each tube 0.25 cc. of heparinized normal rabbit plasma, which was distributed by rotating the tubes in groups of six in pipette rests until clotting was observed. After nutrient medium had been added to each tube, they were stoppered and placed in a roller-tube mechanism (*1*) rotating at 6 r.p.h.

The plasma was obtained as follows: Sterile 1:600 heparin solution (H.W.D.) was placed in a chilled tube in an ice bath, one-hundredth of the volume of blood to be drawn. Blood was then drawn from a rabbit's heart and quickly placed in the tube. Centrifugation was carried out in ice at 2,000 r.p.m. for 6 minutes, the plasma then being drawn off into another chilled centrifuge tube. This tube, if kept in ice, would not show clotting of the plasma for several hours. Within a few minutes of drawing off, however, the plasma was used to coat the tubes. The heparin concentration was such that the change of temperature from that of the ice bath to room

[1] This investigation was conducted under the Commission on Measles and Mumps, Army Epidemiological Board, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.