

Effect of Crystalline Trypsin on the Raw Soybean Growth Inhibitor. (20330)

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In a previous report(1) we showed that trypsin (1-300) fed at 0.15% of the diet neutralized most of the effect of the growth inhibitor present when one-fourth of the protein in the diet of the chick was provided as raw soybean meal. This relative amount of raw meal caused practically as much growth inhibition as did higher proportions. It has been indicated that the substance in crude trypsin, which is able to reverse the action of the soybean growth inhibitor in rat diets, is not trypsin but some associated impurity(2). In a critical test of this question we have fed crystalline lyophilized trypsin in chick diets containing raw soybean protein.

Methods. The procedure was the same as previously described(1). Chicks were carefully selected for uniform size and vitality and then divided equally into pens of 9 each.

Results. The results of 2 experiments are given in Table I. These show a distinct increase in weight gains when the crystalline trypsin was added.

Discussion. It is not certain that the amount of trypsin added was optimal, but this amount was clearly sufficient to demonstrate the anti-inhibitor activity of highly purified

TABLE I. Effect of Crystalline Trypsin on the Growth Inhibition Caused by 5% Raw Soybean Protein in Chick Diets.*

Trypsin† added to 5% raw pro- tein diet, %	Avg wt of chicks, g	
	Exp. 1, 25 days	Exp. 2, 27 days
.00	191.4	206.6
.05	223.9	244.3

* Diets contained a total 20% soybean protein plus 0.3% methionine.

† Worthington Biochemical Sales Co., crystallized, lyophilized trypsin.

trypsin. We have shown that a low level of trypsin may exert no discernible effect, presumably because a low level may be completely inactivated in the presence of a surplus of the antitrypsin known to be present in the raw meal(1). Enough trypsin must be added to neutralize the antitrypsin to the extent that proteolytic enzymes secreted by the animal may be allowed a moderate to complete freedom of action.

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Received May 11, 1953. P.S.E.B.M., 1953, v83.

Application of Thermistor to Measurement of Subcutaneous Temperatures During Hydrothermal Injury in the Rat.* (20331)

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During the course of experiments conducted in this laboratory on the biochemical changes in skin resulting from hydrothermal exposure of the anesthetized rat(1), it became necessary to obtain a continuous recording of the

subcutaneous temperatures in areas of skin exposed at 50°-70°C. The present paper describes an apparatus incorporating a thermistor as the temperature-sensing element which has been used successfully during the past year for this purpose.

Development of the apparatus. In developing a suitable apparatus, it was recognized

* This work performed under contract between the U. S. Atomic Energy Commission and Western Reserve University.

that the temperature-sensing element which was to be placed under the skin must necessarily be very small and must possess a very low thermal capacity in order to permit an immediate and accurate recording of the temperature changes (30° - 70°C) in the surrounding medium. Since a continuous temperature recording was desired, it was imperative that a recording instrument be incorporated.

The application of the thermocouple to the present problem was given first consideration. Extremely small needle thermocouples in which the thermocouple junction is placed in the end of a hypodermic needle can be constructed from commercially available thermocouple wire and stainless steel hypodermic tubing(2-4). Thermocouples, however, have their disadvantages when used for low temperature measurements. The signal resulting from a temperature change of a few degrees is very small, of the order of microvolts. The leads connecting the junctions and the indicating instrument must be of the junction metals. Any inhomogeneity, either of composition or structure, in the leads will produce spurious potentials if subjected to a temperature gradient. Finally, there is the necessity for maintaining a constant reference junction temperature. The accuracy of the thermocouple is no better than that of the temperature of the reference junction.

These drawbacks to the thermocouple directed our attention to the application of other devices of electrical thermometry. For many years the changes in resistance of certain materials, *e.g.*, platinum wire, with changes in temperature have been used as the principle of many thermometric devices. In 1945, a new circuit element, the thermistor, was introduced. The thermistor is a resistor made of a semi-conductor with a negative thermal coefficient of resistance as high as 4.7% per degree C(5). Thermistors are being used extensively for current control and temperature-sensing elements. They are made in a large variety of sizes and shapes. One of the smallest sizes currently available is a glass-covered bead 0.015" in diameter. Its resistance at 20°C is 2000 ohms, which falls to 900 ohms at 50°C . A temperature change from 20° to 50°C will produce a voltage

change of 0.110 volt with only 0.0001 ampere flowing through the thermistor. In contrast, an iron-constantan thermocouple will produce only 0.0015 volt with the same temperature differential. Thus, with the thermistor it is possible to use a much less sensitive indicating element or to eliminate the amplification used with the thermocouples. The meter zero may be set at any temperature up to 150°C by adjusting the balance of the bridge. The necessity for maintaining a reference temperature is obviated. The resistance of the thermistor is high enough that normal changes in the resistance of the leads may be neglected. Any instability resulting from resistance changes of the other resistors in the circuit can be compensated by a variable resistor.

Drummetter and Fastie(6) have successfully applied the thermistor to the measurement of blood temperature and their experiences led us to attempt the adaptation of the thermistor to our needs. Thermistors have also been employed to measure the very small temperature changes occurring in an enzyme-catalyzed reaction(7). Western Electric No. 27A thermistor glass-covered beads, 0.015" in diameter and 0.020" long, with both leads coming from the same end, are employed in the present study. The leads are 0.001" platinum wire and are $\frac{3}{8}$ " long. Thermistors with longer leads are available commercially.[†] To permit insertion under the skin, the thermistor bead is placed in the end of a 4" piece of 24-gauge stainless steel needle tubing (Fig. 1). The lumen at one end of the needle is enlarged with a No. 78 drill (0.016") to receive the thermistor. The needle is then sharpened in the usual manner and the back end of the bevel extended as shown in Fig. 1. After honing the point, removing burrs from the edges and washing to remove abrasive, the point is ready to receive the bead. The lead wires should be coated twice with insulating varnish thinned with benzene. The bead is covered with cement to insulate it from the needle and worked into place, leaving the upper surface of the bead exposed. The cement should not contain a solvent which can

[†]Victory Engineering Corp., Newark, N. J.

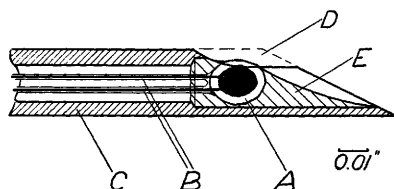


FIG. 1. Diagram of the thermistor needle-type thermometer. A, glass-covered thermistor bead; B, insulated leads; C, needle tubing; D, portion of the needle wall ground off to expose the top of the bead; E, cement holding the bead in position.

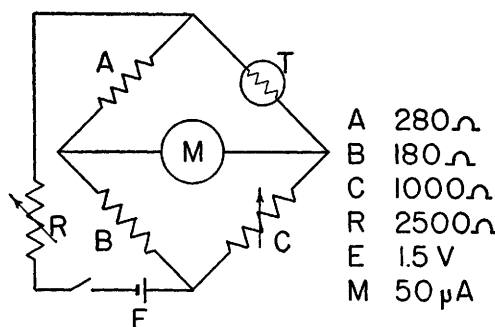


FIG. 2. Wheatstone bridge circuit used with W.E. thermistor No. 27A for measuring temperatures in the range from 35° to 60°C. A and B are selected to give a linear relationship between temperature of the thermistor T and deflection of the meter M. C and R are adjusted for balance and desired sensitivity, respectively.

dissolve the insulation from the wires. A handle may be constructed on the other end of the needle and provision made to connect the wires which go to the bridge.

The relationship between temperature and resistance of a thermistor is non-linear as is the off-balance current of a Wheatstone bridge with respect to changes in thermistor resistance. Using a 1:1:1:1 bridge circuit, these non-linearities compensate adequately for a range of 10°C, yielding a linear meter-temperature curve. Drummeter and Fastie(6) obtained linearity over a 20°C range by using a zero-center meter and measuring 10°C above and below the bridge balancing point. In order to obtain linearity over a greater temperature range with the left-center recording meter available to us, it became necessary to modify the resistance values as shown in Fig. 2. The bridge is balanced at 35°C by means of the variable resistor at C. With the thermistor at 60°C, R is adjusted to give full scale de-

flection. With the zero and full scale readings set as above, the shape of the meter-temperature curve will change with changes in A:T. Further modification may be effected by changing the ratio A:B and the resistance of the meter M. Increasing A:B to 1:10 or more will improve the linearity of meter vs. resistance without necessarily improving the linearity of the meter vs. temperature curve(8). For calibration purposes, the thermistor resistance was measured at a number of known temperatures over the desired temperature range (25°-70°C) by immersing the thermistor in a stirred water bath whose temperature was determined with a National Bureau of Standards calibrated thermometer. In order to facilitate the checking of the linearity of the meter and its calibration, these resistance values could be substituted for the thermistor by means of a decade resistance box.

With the thermistor utilized in our apparatus, the resistance values shown in Fig. 2 give a linear meter deflection from 35° to 60°C within 0.05°C. When a chart record is desired, the meter is paralleled with a G-E photoelectric self-balancing potentiometer millivoltmeter with a sensitivity of 50 millivolts full scale and R adjusted for full scale recording meter deflection. For a more accurate fast response of the millivoltmeter, the microammeter should be replaced with a resistance whose value is that of the resistance of the meter.

The manufacturing tolerances on thermistors are $\pm 25\%$, and if more than one thermistor is to be used with one bridge, compensation may be added to the individual thermistor units by the method described by L. J. Anderson(9). This will match several thermistors at 2 points on the temperature-resistance curve at a slight sacrifice of sensitivity.

The needle-type thermistor thermometer described above has been used extensively in our laboratory to obtain a continuous recording of abdominal subcutaneous temperatures in areas of skin subjected to temperatures ranging from 50° to 70°C for varying periods of time(1). The needle was inserted under

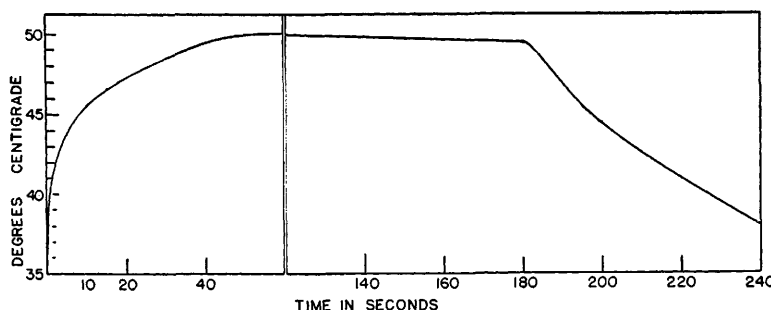


FIG. 3. Subcutaneous temperatures in a rat exposed to circulating water at 55°C. The rat was placed in water bath at $T = 0$ sec. and removed at $T = 180$ sec.

the skin before exposure, its position being verified by dissection following the thermal episode. A typical example of a subcutaneous temperature curve is shown in Fig. 3.

Summary. A simple and sensitive thermoelectric device for the measurement of temperature has been described which incorporates the thermistor as the temperature-sensing element. As a needle-type thermometer, this apparatus has been used extensively in our laboratory to obtain continuous recordings of abdominal subcutaneous temperatures attained during the production of hydrothermal burns in rats. On the basis of the many advantages inherent in this apparatus, its further utilization for temperature measurements in biological studies is advocated.

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Received May 12, 1953. P.S.E.B.M., 1953, v83.

Resistance to Folic Acid Analogues in a Strain of *Streptococcus faecalis*.^{*} (20332)

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The possibility that organisms which are resistant to certain antagonists of folic acid

possess or develop the ability to convert such analogues to metabolically active compounds has formed an attractive hypothesis. Aminopterin (4-amino-pteroylglutamic acid) is readi-

^{*} This investigation was supported, in part, by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service, and, in part, by a grant from the Lederle Laboratories Division of the American Cyanamid Co.

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[§] We are indebted to Dr. Joseph H. Burchenal and Dr. Dorris J. Hutchison for cultures of the antagonist-resistant strains of *S. faecalis* and *Leuconostoc citrovorum*.