a sheep with these combined factors—virus and enteric toxin[9]—and recently we have produced paralysis in a horse by injecting these combined elements.

JOHN A. TOOMEY

HEMORRHAGIC NECROSIS AND REGRESSION OF SARCOMA 180[1]

BACTERIAL substances capable of eliciting the phenomenon of local skin reactivity to bacterial filtrates in rabbits[2] produce, upon intravenous injection, hemorrhagic necrosis and regression of transplantable malignant tumors of guinea pigs,[3] mice and rats.[4] There is, however, a high death rate in animals thus treated.

In experiments on the phenomenon of local skin reactivity to bacterial filtrates in rabbits it was observed that, in certain proportions, mixtures of *B. typhosus* culture filtrates with homologous antisera possess a high phenomenon-producing and low lethal potency. Studies were then made on the effect of single intravenous injections of these mixtures and of toxic filtrates alone upon 132 mice bearing twelve days old sarcoma 180. Best results were obtained with a mixture of 300 *B. typhosus* reacting units with 200 neutralizing units of antityphoid horse serum[5] tested in 27 mice. There was no early mortality (*i.e.*, 24 hours after the intravenous injection). Late mortality (*i.e.*, 2 to 20 days after the intravenous injection) occurred in 5 mice. Prompt hemorrhagic necrosis took place in 23 mice and complete regression of tumors with uneventful healing in 21 of these mice. According to Woglom,[6] untreated mice show only 1.33 per cent. of spontaneous regressions. As the amount of filtrate in mixture with 200 neutralizing units of the serum was increased, there occurred early mortality and a roughly proportionate rise in late mortality. The incidence of complete regressions of tumors of surviving mice was approximately the same. Doses of 125 and 250 reacting units of the filtrate alone (*i.e.*, without the serum) elicited early mortality as high as 70 and 95 per cent., respectively.

It becomes obvious from these experiments that in certain proportions mixtures of *B. typhosus* filtrates with homologous antisera possess a comparatively low lethal potency and yet elicit prompt and intense hemorrhagic necrosis with subsequent complete regression of sarcoma 180 in a high percentage of mice well above normal expectancy. Further work is under way in order to determine the effect of these and other combinations of bacterial filtrates with immune antisera upon animal and human spontaneous tumors.

GREGORY SHWARTZMAN

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A THYRATRON CONTROL FOR INCUBATORS AND WATER BATHS

THE use of an electromagnetic relay for the control of heating elements in incubators and water baths has two fundamental disadvantages. (1) The current required to operate even highly sensitive relays is more than is desirable at the mercury platinum junction of the thermoregulator. This can be corrected by the use of an amplifying vacuum tube, but adds to the complexity of the set-up without avoiding the second disadvantage. (2) Any relay, no matter how well made, is apt to stick and possibly ruin an extensive experiment. This can be avoided by using a duplicate relay and thermoregulator, but again the system is complicated. These disadvantages are obviated by the use of a thyratron to replace the amplifying tube and relay. For this purpose we have used a General Electric Thyratron, FG154, which is a four-electrode, Argon-filled, low-grid current control tube. This tube is rated to carry a current of 2.5 amps. continuously, which is sufficient for the temperature control of most incubators and the average water bath. Under ordinary conditions, with currents not exceeding the maximum rated value, a tube may be expected to serve for at least one year. Another tube, FG98, capable of carrying .5 amps., is available for smaller incubators or temperatures near to that of the room.

The figure gives a wiring diagram of the set-up used. The tube has six terminals, four which fit into the base and are lettered F, F, P and G. F and F connect with the ends of the filament and P with its midpoint. G connects with the shield grid. The terminal of the control grid is at the side of the tube and the terminal of the anode at the top. The heating unit may be in any desired form. The filament transformer must be capable of delivering 35 watts at 5.0 volts A.C., and the filament current should be as nearly constant as possible to ensure a long life for the tube. The two batteries may be dry cells. The current drawn from them is so small that they may be expected to last approximately their "shelf life."

[9] *Idem, Proc. Soc. Exp. Biol. and Med.*, 32: 1346, 1935.

[1] From the laboratories of The Mount Sinai Hospital, New York City. Preliminary report. This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[2] G. Shwartzman, *Jour. Exp. Med.*, 48: 247, 1928.

[3] Gratia and Linz, *C. R. Soc. Biol.*, 108: 427, 1932.

[4] G. Shwartzman and N. Michailovsky, *Proc. Soc. Exp. Biol. and Med.*, 29: 737–741, 1932; D. Duran-Reynals, *Proc. Soc. Exp. Biol. and Med.*, 31[1]: 341, 1933–34; K. Apitz, *Ztsch. f. Krebsforst.*, 40[1]: 50, 1933.

[5] G. Shwartzman, *Jour. Exp. Med.*, 52: 781, 1930.

[6] William H. Woglom, personal communication.

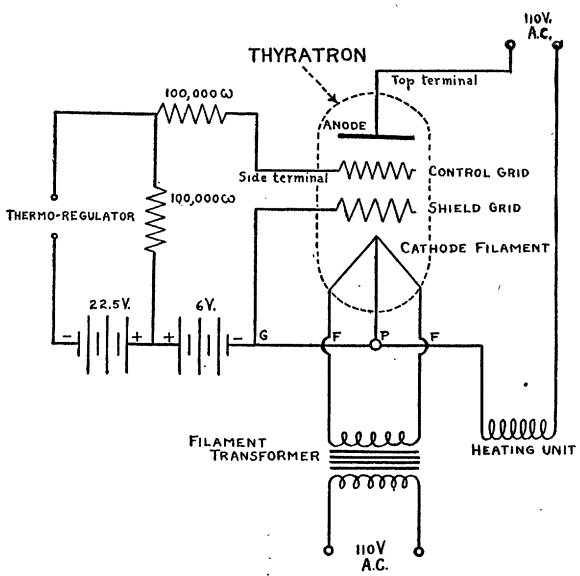

FIG. 1. Wiring diagram for thyratron control for incubators and water-baths.

The 100,000 ohm resistances are of the type commonly used for grid leaks in radio sets. The thermoregulator may be of any make-and-break type. The wiring diagram shown is such that when contact is made at the thermoregulator, the heating current is off and *vice versa*. Care should be taken that the filament current is turned on some fifteen seconds before the heating current comes into the circuit.

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THE PREPARATION OF BIXIN

THE dried seeds of *Bixa orellana*, a plant native to the West Indies, are known to contain much of the red pigment, bixin, as well as a little carotene and other materials.

Attempts to isolate this bixin by methods devised by Riffart,[1] Heiduschka and Panzer,[2] and van Hasselt[3] were quite unsuccessful in our laboratory, giving a very small yield. In order to obtain a good yield of pure crystalline material with the minimum of manipulation, the following method was developed:

A several hundred gram portion of Jamaica annatto seeds was ground in a nut-meat grinder and then allowed to stand in a large, wide-mouth, loosely corked bottle for two days to allow the coarse meal to dry. This coarse material was then further ground to a fine powder in a large laboratory mill and sifted through a 60-mesh sieve.

[1] Riffart, Dissert., München, 1911.
[2] A. Heiduschka and A. Panzer, *Ber.*, 50: 546, 1917.
[3] van Hasselt, Dissert., Delft, 1910.

About 250 grams of this pulverized annatto was treated with a liter and a half of cold acetone in a three-liter flask for fifteen to twenty minutes, the mixture being agitated every few minutes. The deeply colored (red-brown) acetone solution was then decanted and discarded because of the large amount of oily and resinous material that it contained. The remaining solid was extracted repeatedly with one-liter portions of boiling acetone until the color of the solid residue was a pale yellow when dry. The combined acetone extractions were filtered and then evaporated to about one tenth their former volume, whereupon there was precipitated a mass of metallic, lustrous purple crystals. With a sufficiently large Soxhlet extraction apparatus, the rather large total volume of acetone was much diminished. (The acetone reclaimed in the process of evaporating the decanted and filtered acetone solution may be used in further extraction of the raw material.)

The final volume, now one tenth of the total volume, was cooled and the precipitated crystalline material filtered therefrom. This crystalline material was crude bixin. It was suspended in about a liter of purified dry acetone and refluxed for a period of an hour in order to saturate the acetone at the boiling point. The hot solution was quickly filtered through a hot-water funnel to remove the undissolved material, and the resulting filtered solution rapidly cooled, first at room temperature and then in an ice-salt mixture. The resulting crystals were deep purple in color and possessed a bright metallic luster. Microscopic examination showed individual crystals to be rhombic in shape and a deep red color by transmitted light. When slowly heated, the crystals melted at 192° C. Treated with concentrated H_2SO_4, the crystalline material, even minute traces, dissolved to give a blue solution. The crystalline material was readily soluble in alkali. The crude bixin, which was about 4.5 per cent. of the annatto powder, was converted into purified bixin crystals with a yield of about 3.5 per cent.

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