# Physiological Studies on Tumor-Inhibiting Agents

## III. Effect on Apparent Systolic Blood Pressure in Mice of the *Serrotia marcsecens* Tumor-Necrotizing Polysaccharide of Shear*†

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Patients treated with *Serratia marcescens* tumor-necrotizing polysaccharide show pronounced hypotension, lasting sometimes for days (3, 5, 6). It was of interest to determine whether a similar reaction occurs in tumor-bearing mice injected with this substance, especially since such mice exhibit reactions suggestive of circulatory disturbances, *i.e.*, prostration and depressed rectal temperature.

The procedure employed by Sobin (8) for measuring systolic blood pressure in the rat has been adapted by us to the mouse. Fig. 1 is a diagram of the apparatus we have employed. The tail-warmer plethysmograph and the occluding cuff were made of pyrex glass.[1] The inner (tail-containing) chamber of the plethysmograph had an inside diameter of 8 mm. and an overall length of 85 mm. Ureteral drainage tubing of 7.5 mm. diameter was tied into place in this chamber, care being taken that the rubber was neither too loose nor too taut. The left-hand opening was closed with a cork, leaving the right-hand opening free for insertion of the tail. The occluding cuff had openings 9 mm. in diameter. Drainage tubing of 10 mm. diameter was tied into place in this cuff. The length of tail occluded by the cuff was 15 mm. The capillary-indicating tube used to read changes in the volume of the tail had an inside diameter of 0.3 mm.

A smaller diameter-indicating tube is needed for the mouse than for the rat, and changes in the level of the meniscus are so small as to require that a small magnifying glass be placed in front of the meniscus. The indicator fluid used by Sobin was unsatisfactory in the smaller indicating tube used by us. One per cent potassium dichromate, plus 0.015 per cent aerosol to reduce surface tension, proved satisfactory. It was of course necessary to make sure that no air bubbles were present anywhere between stopcock 1 and the top of the column of dichromate in the capillary indicating tube.

The middle part of the mouse holder consisted of a cylinder, open at both ends. This cylinder was made of lumerith, a transparent cellulose acetate product sold in sheet form, which can be easily bent and cut. Any two surfaces can be molded together by placing a small drop of acetone between them and holding them firmly together for a moment. Lumerith sheets of 0.2 mm. thickness were used, into which were punched many small holes, so that the mouse would not become overheated. Since mice can easily chew their way through lumerith, the head end of the holder was made of a metal cap, long enough to contain the entire head of the mouse. A 0.5 cm. hole was drilled in the end of the cap to provide an air supply for the mouse. The metal cap and the middle of the holder were bound firmly together with cellophane tape. The tail end of the holder consisted of a cap made of lumerith, in the end of which a hole about 1 cm. in diameter was cut, through which the tail could project. The tail end of the holder was so made that it would slip easily but snugly over the open end of the middle part of the holder. It is usually an easy matter to induce a mouse to crawl into the opening in the middle part of the holder. With the mouse in place, the middle and tail parts of the holder were sealed together with cellophane tape.

With the mouse in the holder the tail was threaded through the occlusion cuff and into the space within the rubber membrane in the inner chamber of the plethysmograph. Threading of the tail into the plethysmograph was facilitated by closing stopcock 2, opening stopcock 1, pulling on the syringe and then closing stopcock 1. This opened up a wide passageway into the tail chamber. Stopcock 1 was then opened and the syringe pushed down somewhat to bring the rubber into contact with the tail.

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† In the first paper in this series (Temperature Changes Produced in Mice with S. Marcescens Polysaccharide—Approaches to Tumor Chemotherapy, A. A. A. S., Washington, D. C., 1947, page 265), please read μg. (*micrograms*) wherever the term mg. appears.

[1] The tail-warmer plethysmograph and the occluding cuff were prepared by Mr. Thomas Walton, 4583 G. St., Philadelphia 20, Pa.

The tail was warmed at 42° to 43° C. for at least 10 minutes, usually 15 to 20 minutes. To take a reading the mercury was pumped up to 200 mm., stopcock 3 being closed. With stopcock 2 closed, pressure was placed on the syringe to push dichromate solution into the space between the glass and the rubber tubing and thus force a small amount of blood out of the tail. Immediately stopcock 3 was opened, occluding the tail at a time when it contained a minimum of blood. Stopcocks 1 and 2 were opened and the syringe moved until the dichromate meniscus in the capillary tube occupied an intermediate position. Stopcock 1 was then closed again, and the mercury in the sphygsomanometer allowed to fall slowly. The systolic pressure was read as that pressure where the meniscus showed a steady permanent rise in the capillary tube. With practice it was usually possible to distinguish this change from changes in the meniscus due to struggling of the mouse. In cases of doubt, readings were repeated. After two or three successive readings had been obtained on a given mouse the mouse was removed and released from the holder. When two successive readings were within 5 mm. of each other they were averaged. When they were further apart than 5 mm., a third reading was taken, and the systolic blood pressure was taken as equal to the average of the two highest values obtained. If no two readings within 10 mm. of each other were obtained, additional readings were made or the data were discarded.

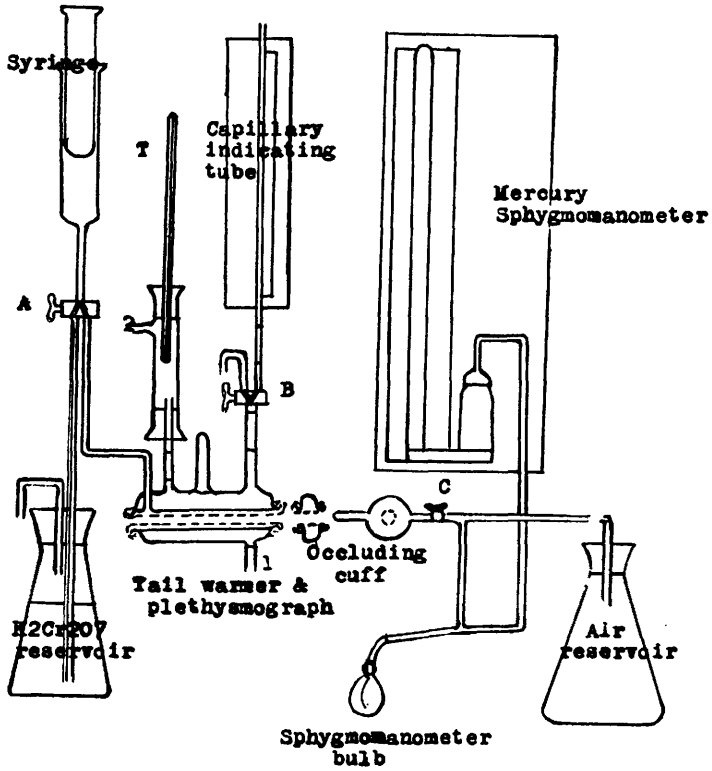


FIG. 1.—Apparatus employed in securing apparent systolic presure readings.
A and B = Two way glass stopcocks.
C = One way metal stopcock.
1 = Point of entrance of hot water into heating jacket of tail-warmer plethysmograph.
2 = Point of exit of hot water from chamber for indicating temperature of plethysmograph.
T = Centigrade thermometer.

The readings so obtained have not been compared with those obtainable on cannulation of a large artery. We have been primarily interested in determining whether *changes* in blood pressure occur in tumor-bearing mice injected with polysaccharide. Even for the purpose of judging whether changes in apparent systolic blood pressure had occurred the method was found to have definite limitations. The making of the systolic pressure determinations was found to produce definite injury to the tail, so that ordinarily it was not possible to secure more than three to six sets of readings on a given mouse. This injury was manifested: (a)

by decreased extent of movement of the dichromate meniscus and increased difficulty in securing readings several hours after the initial reading, even in mice not treated with polysaccharide and not showing any appreciable change in the apparent systolic blood pressure. These effects were noted on mice in which attempts were made to secure readings at hourly to half-hourly intervals, but were infrequent in mice used to obtain readings at 2 hour intervals, (b) by frequent failure to secure readings on the subsequent day in mice that were quite lively and showed normal rectal temperatures (c) by reddening of the tail and occasional rubbing off of part of the skin (d) by definite necrosis and falling off of part of the tail, occurring within the next day or so after the initial readings. Reactions such as those described above have been reported by Sobin (8) for young rats only.

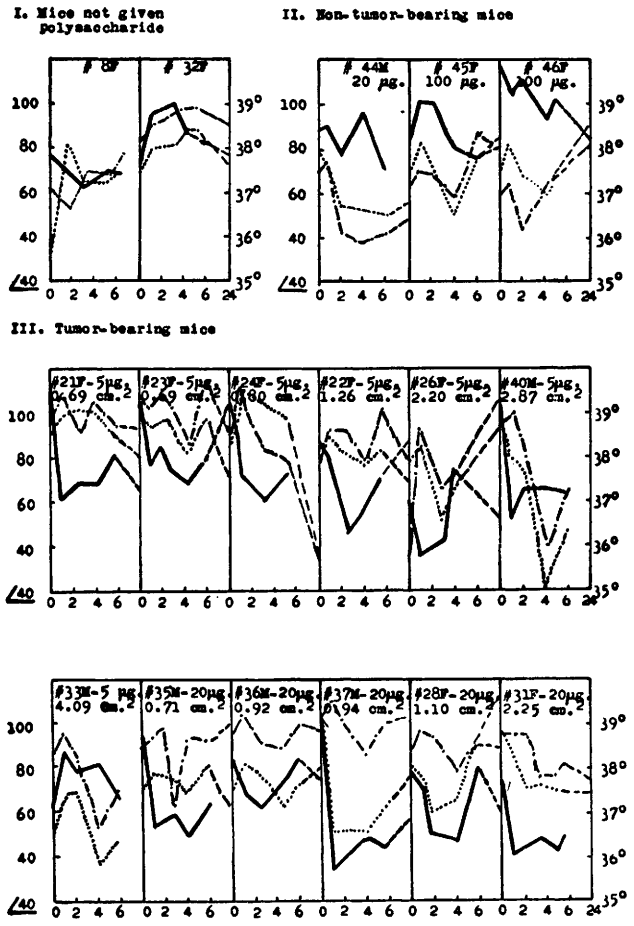


FIG. 2.—Changes in apparent systolic pressure and in rectal temperature in mice following intraperitoneal injection of *Serratia marcescens* polysaccharide preparation $P_8$.

Left-hand abscissæ and solid lines = Systolic pressure in mm. Hg.

Right-hand abscissæ = Rectal temperatures in degrees centigrade.

Dotted lines = rectal temperatures after S.P. determinations.

Dot-dash lines = Rectal temperatures after S.P. determinations.

Dash lines = To connect 6 hour readings with 24 hour readings (0 hours for one mouse equals 24 hours for preceding mouse).

Ordinates = Time in hours after injection of polysaccharide. Zero (0) times refer to readings obtained before polysaccharide injection.

Injection of polsyaccharide was followed by definitely lower systolic blood pressure readings. The lower these readings, the smaller was the movement of the dichromate meniscus. This was a limiting factor which prevented our securing a reading when the apparent systolic blood pressure was below 40 mm. Hg. Where we felt that injury of the tail had not been sufficient by itself to prevent the obtaining

of a reading we have designated failure to obtain a reading by the symbol /40 to indicate an apparent systolic pressure of less than 40 mm. Hg.

In the mouse, injection of polysaccharide is followed not only by fall in apparent systolic pressure but also by decrease in rectal temperature (2). The tail warming method of determining systolic blood pressure depends on the securing of maximum dilatation of tail arterioles as a result of warming the tail to 42° to 43° C. It is questionable whether maximum dilatation occurs in mice having definitely subnormal rectal temperatures (see Discussion). It should be noted here that following polysaccharide injection the fall in apparent systolic pressure in the mouse occurs some time before any appreciable change in rectal temperature has occurred, so that early systolic blood pressure readings by the tail method are probably closer to the systolic blood pressure in the large arteries near the heart than is the case for readings obtained later, when the rectal temperature is definitely depressed. Using thermocouples, we have made rectal temperature readings before and after each set of systolic blood pressure readings.

## EXPERIMENTAL FINDINGS

Figure 2 shows typical systolic pressure and rectal temperature readings obtained on Carworth Farms white mice treated with polysaccharide (preparation $P3)_3$. These data, and data obtained on additional mice, are summarized in Table I. Tumors (Sarcoma 37) of cross-sectional area less than 0.5 sq. cm. weighed less than 0.1 gm; those with cross sectional areas greater than 0.5 sq. cm. had weights ranging up to 2.5 gm. Plus, minus figures in the Tables represent standard errors.

Tables II and III summarize data obtained following injection of 20 micrograms per mouse of polysaccharide (preparation $P3)_3$. Only three sets of systolic pressure readings were made on a given mouse, since it was thought this should decrease the likelihood of securing readings that were grossly erroneous because of injury to the tail. When a mouse died on the experimental day the tumor was removed and weighed that day. Otherwise the tumor was secured and weighed on the following day.

TABLE I: CHANGES IN APPARENT SYSTOLIC BLOOD PRESSURE AND IN RECTAL TEMPERATURE IN MICE FOLLOWING INTRAPERITONEAL INJECTION OF *Serratia marcescens* POLYSACCHARIDE PREPARATION P[a]

| External Area of Tumors: (Mouse Sarcoma 37) | | Zero (no tumor) | | Less than 0.5 sq. cm. | More than 0.5 sq. cm. | |
|---|---|---|---|---|---|---|
| Dose of polysaccharide: (gm. per mouse) | | 20 | 100 | 5 | 5 | 20 |
| No. of mice in group: | | 3 | 2 | 9 | 8 | 6 |
| Mean S.P.* before polysaccharide injection (mm. Hg.) | | 89 | 100 | 91.7 ±5.3 | 90.9 ±6.8 | 95.3 ±7.3 |
| Changes in S P. during 1st 6 hrs. after polysaccharide injection: (in mm. Hg.) | Av. for all readings all mice in group: | − 4.7 | − 3.5 | −14.2 ± 4.1 | −25.9 ± 4.7 | −40.2 ± 9.5 |
| | Av. for lowest S.P. value for each mouse: | −14.3 | − 15.0 | −29.2 ± 4.1 | −41.0 ± 2.3 | −50.3 ±11.0 |
| Av. of the times for each mouse at which the lowest S.P. reading was secured: (in hours) | | | | 3.4 ± 0.40 | 2.6 ± 0.71 | 3.1 ± 0.79 |
| Changes in rectal temp. during 1st 6 hrs. after polys. inj. (in C.) | Av. for all readings on all mice in group: | − 0.53 | 0.11 | − 0.32 ± 0.38 | − 0.05 ± 0.33 | − 0.68 ± 0.36 |
| | Av. for lowest reading for each mouse: | 0.73 | − 0.53 | − 0.85 ± 0.11 | − 0.74 ± 0.48 | − 1.40 ± 0.39 |
| Av. of the times for each mouse at which the lowest rectal temperature reading was obtained (hours): | | | | 3.50 ± 0.38 | 4.36 ± 0.32 | 4.50 ± 0.40 |

*For all tumor-bearing mice given polysaccharide†*

Av. time to reach lowest S.P. reading $-2.91 \pm 0.36$ hours

Av. time to reach lowest rectal temp. $-3.81 \pm 0.24$ hours

S. P. = Systolic pressure.

From Fig. 2 and Tables I and II the following conclusions may be drawn:

A. Intraperitoneal injection of *Serratia marcescens* polysaccharide into tumor-bearing mice in amounts sufficient to cause considerable hemorrhage and necrosis in the tumor is followed within an hour or so by a definite to profound decrease in apparent systolic pressure.

B. Mice injected with polysaccharide usually show a decreased rectal temperature. This decrease generally occurs after the fall in systolic pressure, but was not always noted, even when a fall in systolic pressure had occurred.

C. The greater the amount of tumor tissue in a mouse the more profound and long-lasting was the decrease in both the apparent systolic pressure and the rectal temperature.

[a] We are indebted to the Chemotherapy Section, National Cancer Institute, Bethesda 14, Md., for samples of *Serratia marcescens* polysaccharide preparations P3 and P8.

D. The greater the amount of tumor tissue in a mouse the greater was the amount of prostration and diarrhea exhibited, and the greater was the likelihood that death would occur subsequent to administration of polysaccharide. This confirms Shear (7).

## DISCUSSION

When the data on rectal temperatures shown in Table II were analyzed to secure Table III some interesting facts were brought to light. In non-tumor-bearing mice, and in untreated mice with tumors weighing less than two grams the rectal

TABLE II: EFFECTS OF 20 MICROGRAMS PER MOUSE OF *Serratia marcescens* POLYSACCHARIDE PREPARATION $P_8$ ON APPARENT SYSTOLIC BLOOD PRESSURE AND RECTAL TEMPERATURE IN MICE BEARING TUMORS (SARCOMA 37) OF VARYING SIZES

| Mouse No. and sex | | Wt. of tumor (gm. | S.P. readings (mm. Hg.) Control | 2 hr. value | 4 hr. value | Rectal temperature readings (°C.) Control values B | Control values A | 2 hour values B | 2 hour values A | 4 hour values B | 4 hour values A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 112 | F | Zero | 123 | 76 | 96 | 37.8 | 38.4 | 37.1 | 37.5 | 38.5 | 38.5 |
| 116 | F | " | 70 | 82 | 50 | 36.9 | 39.2 | 38.1 | 39.5 | 38.2 | 38.9 |
| 117 | F | " | 90 | 90 | 88 | 37.4 | 38.4 | 36.0 | 38.0 | 37.6 | 38.5 |
| 118 | F | " | 104 | 75 | 65 | 37.9 | 38.6 | 36.6 | 39.4 | 38.1 | 38.1 |
| 233 | F | " | 100 | 70 | 90 | 38.0 | 38.8 | 36.0 | — | 37.7 | — |
| 83 | F | 0.04 | 95 | 83 | 81 | 39.0 | 38.4 | 36 8 | 37.9 | 38.1 | 36.8 |
| 79 | F | 0.12 | 103 | 62 | 63 | 37.7 | 38.6 | 37.5 | 38.5 | 38.3 | 37.2 |
| 100 | F | 0.16 | 103 | 91 | 83 | 37.1 | 37.4 | 36.0 | — | 35.6 | 34.4 |
| 80 | F | 0.17 | 134 | /40 | 78 | 37.7 | 38.0 | 35.7 | — | 34.0 | 33.9 |
| 77 | F | 0.30 | 106 | 61 | 80 | 37.6 | 38.8 | 38.0 | 37.8 | 37.0 | 37.7 |
| 106 | F | 0.30 | 97 | 56 | 70 | 37.8 | 38.9 | 37.0 | 37.3 | 38.5 | 38.0 |
| 76 | F | 0.38 | 80 | 42 | /40 | 38.7 | 39.3 | 37.9 | 38.0 | 35.0 | 38.1 |
| 130 | M | 0 39 | 100 | 73 | /40* | 39.1 | 39.2 | 37.1 | — | 34.6 | Dead |
| 177 | F | 0.60 | 80 | 66 | 73 | 37.5 | 39.3 | 38.0 | 37.6 | 33.8 | 33.4 |
| 75 | F | 0.69 | 103 | 76 | 46 | 38.1 | 39.3 | 38 3 | 37.3 | 37.5 | 37.3 |
| 169 | F | 1.05 | 80 | 85 | 60 | 35.7 | 37.6 | 34.8 | 35.3 | 34.9 | 35.1 |
| 247 | F | 1.05 | 120 | 40 | 50 | 35.6 | 37.9 | 35.4 | 36.6 | — | 35.9 |
| 248 | M | 1.10 | 66 | 63 | /40† | 38.0 | 39.0 | 38.2 | 39.3 | 34.5 | 34.4† |
| 173 | F | 1.35 | 111 | 51 | 42 | 38.9 | 38.5 | 37.3 | 37.7 | 33.4 | 33.5 |
| 176 | F | 1.40 | 48 | 60 | 59† | 37.0 | 38.7 | 38.4 | 39.2 | 34.5 | 35.1† |
| 174 | F | 1 73 | 89 | 90 | 55 | 38.1 | 39.3 | 38.3 | 37.3 | 37.5 | 37.3 |
| 245 | F | 2.50 | 82 | 67 | 50 | 38.6 | 38.5 | 38.1 | 37.6 | 37.2 | 37.3 |
| 121 | M | 2.51 | 72 | /40 | /40 | 37.9 | 37.9 | 37.1 | 37.6 | 35.3 | 33.8 |
| 105 | F | 2.59 | 121 | 52 | 45* | 38.0 | 39.0 | 36.3 | 35.6 | 33.9 | 35.1* |
| 181 | F | 2.70 | 80 | 52 | /40 | 38.3 | 38.7 | 36.6 | 35.7 | 36.4 | 35.5 |
| 107 | F | 2.78 | 110 | 80 | Dead | 36.7 | — | 34.8 | — | Dead | |
| 201 | M | 3.50 | 72 | 75 | /40 | 36.9 | 37.3 | 37.9 | 37.1 | 34.0 | 34.4 |
| 140 | F | 3.53 | 56 | 62 | /40† | 38.2 | 37.4 | 38.1 | — | 32.1 | 32.1† |
| 228 | F | 3.66 | 50 | /40 | /40* | 36.1 | 35.7 | 35.9 | 36.3 | 32.6 | 32.1† |
| 250 | F | 3.80 | 80 | 90 | /40† | 37.7 | 37.5 | 36.4 | 36.1 | 35.7 | —† |

* Mouse died soon after reading was made
† Mouse found dead next morning
/40: No reading obtained—apparent systolic blood pressure less than 40 mm. Hg.
B: rectal temperature readings obtained *before* corresponding systolic pressure readings.
A: rectal temperature readings obtained *after* corresponding systolic pressure readings

E. As compared with tumor-bearing mice, normal mice showed much smaller changes in apparent systolic pressure and rectal temperature following injection of polysaccharide, even when considerably bigger doses were given. The dose of polysaccharide (preparation P8) required to kill over half of normal Carworth Farm mice is of the order of 500 micrograms per mouse, as compared with a dose of 20 micrograms for mice bearing tumors weighing more than 2 grams.

F. Mice with large tumors (1 gram or more) were more likely to show low original systolic pressure readings and low rectal temperature readings than either normal mice or mice with smaller tumors.

temperatures obtained after taking the systolic pressure readings were on the average nearly 1°C. higher than the corresponding rectal temperatures which had been obtained on these same mice prior to the tail heating which preceded the taking of the systolic pressure readings. This increase in rectal temperature is to be expected if an appreciable amount of heat is carried from the heated tail to the body of the mouse by the blood flowing through the tail. Conversely, the usual failure to secure such a rise in rectal temperature in mice with tumors of weight more than two grams indicates a sluggish blood flow through the tail in these mice.

Generally, an appreciable rise in rectal temperature, in connection with the tail heating, did not

occur in tumor-bearing mice at 2 and 4 hours after administration of polysaccharide, or in non-tumor-bearing mice at 4 hours after administration of polysaccharide. This indicates that a sluggish flow of blood in the tail is induced by the polysaccharide, particularly in tumor-bearing mice. The only other way in which this effect could be produced would be by a marked increase in rate of heat loss in the polysaccharide treated mice. This is very unlikely, since in mice administration of polysaccharide results in depression of both rectal temperature (2) and skin temperature (unpublished). Furthermore, Algire (1) has found that a sluggish blood flow and a marked decrease in functional vascular level occur in both sarcoma 37 and skeletal muscle following administration of the polysaccharide to mice prepared with a transparent chamber for microscopic observation.

Table III: Values Obtained When Rectal Temperature Readings Taken Just Before Systolic Pressure Readings are Subtracted from Corresponding Rectal Temperature Readings Obtained Just After These Same Systolic Pressure Readings

| Group of mice | No. of mice in group | Control values | 2 hr. values | 4 hr. values |
|---|---|---|---|---|
| Normal mice | 4 | 0.90 | 1.18 | 0.40 |
| Mice with tumors of wt. 0.04–1.73 gm. | 12 | $0.97 \pm 0.23$ | $0.23 \pm 0.22$ | $0\ 09 \pm 0.20$ |
| Mice with tumors of wt. 2.50–3.80 gm. | 7 | $0.06 \pm 0.20$ | $0.19 \pm 0.24$ | $0.17 \pm 0.34$ |

Decrease in ability of the tail to convey heat to the body of the mouse, cold skin, lowered rectal temperature, and the observations of Algire all indicate that appreciable peripheral vasoconstriction occurs following administration of polysaccharide. The heated tail method of securing systolic pressure readings depends for its reliability on the securing of complete arterial and arteriolar relaxation in the tail as a result of heating the tail to 42° to 43° C. It would appear unlikely that such relaxation occurs in polysaccharide treated mice; the readings in such mice may be expected to be appreciably lower than they would have been if complete arteriolar relaxation in the tail had been secured. Nevertheless we feel that the evidence is in favor of a generalized fall in blood pressure being produced in mice by polysaccharide treatment, since in most of the readings taken within 2 hours after polysaccharide administration, in which an apparent lowering of systolic blood pressure occurred, there was an accompanying increase in rectal temperature, subsequent to tail-heating of at least 0.5° C. (*See* Nos. 40, 33, 35, 36, 37, 28 and 31 of Fig. 1).

The definite correlation of the apparent systolic blood pressure readings with: (a) the degree of prostration and diarrhea exhibited by these mice; (b) the rectal temperature readings; and (c) the likelihood of death occurring subsequent to injection of polysaccharide indicate that apparent systolic blood pressure readings as obtained by the heated-tail method may be used as an objective criterion of the physiological condition of the mouse, even though they are probably of little value in judging the absolute level of systolic pressure in large arteries near the heart in tumor-bearing, polysaccharide-treated mice.

The injury to the tail of the mouse produced by the heated-tail method of securing systolic pressure readings is a matter of some interest, especially since it is apparently much less likely to occur in the rat. The mouse has a very high metabolic rate in relation to *body weight* (4). Heating of the tail to 42° to 43° C. may be expected to raise the metabolic rate in the tail still further. It seems to us likely that the tail injury produced by the procedures employed in taking systolic pressure readings is due to severe anoxia. If we take Herrington's values (4) for the rat, and convert these into values *per kgm. of body weight* we find that the metabolic rate for a 200 gm. rat is only about 40 per cent of that of a 20 gm. mouse, and the metabolic rate of a 100 gm. rat is about 50 per cent of that of a 20 gm. mouse. From this we would expect that the procedures involved in securing systolic pressure readings in a heated-tail would be more likely to produce irreversible damage by anoxia in the mouse than in the rat.

## SUMMARY

1. A method is described for securing systolic blood pressure readings in the heated tail of the non-anaesthetized mouse.

2. These readings were depressed in Carworth Farms white mice injected with *Serratia marcescens* tumor-necrotizing polysaccharide.

3. The extent of the depression was correlated with the amount of tumor tissue (Sarcoma 37) in the mouse, and with the dose of polysaccharide given.

4. The question as to whether the depression in apparent systolic pressure was due primarily to a generalized fall in blood pressure or to vasoconstriction in the tail is discussed.

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