BIO-ELECTRIC CORRELATES OF METHYL-COLANTHRENE-INDUCED TUMORS IN MICE*

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Previous studies have shown the presence of definite changes in the bio-electric pattern of mice correlated with the onset and presence of spontaneous adenocarcinoma of the mammary gland (Burr, Smith, and Strong, 1938). It was found that the arrangement of bio-electric gradients was characteristic for each of the two strains of mice used; one being cancer-susceptible, the other relatively cancer-resistant. The age of the animal, likewise, seemed in part to contribute to this pattern. In young mice a marked rise in potential gradient across the chest was noted antecedent to recognition of the tumor by palpation. In middle-aged mice the voltages between the same two points tended to be more variable than in the controls, while in old mice a sharp rise in potential was noted following the obvious appearance of the tumor mass. All these facts strongly suggest that the presence of spontaneous new growth modifies in characteristic fashion certain of the bio-electric gradients in mice.

The present report deals with a similar study of new growth in mice induced by subcutaneous injection of methylcolanthrene dissolved in sesame oil. To this end, 40 mice, 6 weeks old, were selected from the Strong CBA strain. These were divided into three groups. Methylcolanthrene in sesame oil was injected into the right axillary space in 20. In 10, sesame oil alone was injected into the same region. The remaining 10 were used as controls. The bio-electric determinations were made with the Burr-Lane-Nims technic, as described in previous papers.[1] The animals were removed from food in the early morning and in the early afternoon were placed on the operating board. The animal was held immobilized by cords attached to the legs; no anesthesia was necessary. Potential gradients were measured between the sternum and the symphysis, from the sternum to the right chest, from the sternum to the left chest, and from the right chest to the left chest. These points were selected because in the previous studies they had seemed to be the most pertinent. The hair over these points was shaved and the area of the skin moistened with salt solution. Glass tubes

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containing physiological salt solution and leading from the silver-silver chloride electrode chambers were then brought to rest on these points on the skin. The voltage difference appearing between each pair of points was then recorded. For the first two months readings were taken every two weeks. Thereafter, to the end of the experiment some three months later, determinations were made every week. Slight inflammatory thickenings in the region of the injection were noted in all animals receiving methylcolanthrene and sesame oil and in a number receiving sesame oil alone. When the tumor was discovered by palpation in the right axilla or in its immediate vicinity, an additional reading was made of the potential gradient between the sternum and the new growth. Of the 20 experimental animals receiving methylcolanthrene, 16 developed obvious tumors, of the remaining four, one showed an epidermal nodule which could not be diagnosed with certainty, a second animal was lost through accident, and two were lost by intercurrent disease. None of the animals receiving sesame oil alone showed any sign of new growth, nor did any of the controls.

In general, all the animals in both experimental groups and in the control group showed a decreasing variability of potential difference during the course of the experiment. This was probably due in part to improving practice in the technic and possibly to the growing adaptation of the animal to handling. However, the degree of week-to-week variability of the readings was rather extraordinary, the correlations between any successive readings being .08. It seems perfectly clear that a good many factors enter into the potential pattern, most of which are not understood. This means that any one factor which might be expected to change the electrical pattern would have to produce a very considerable bio-electric effect in order to be detected. However, in spite of this variability, certain findings seem of interest.

While the week-to-week change in potentials seems to be great, it appears to fall within certain well-defined limits. If one compares the variability during the first five weeks of the experiment with the variability of the last five weeks, certain significant differences are to be seen. In the control group variability at the beginning of the experiment averaged 2339 uv. During the later part of the experiment, the variabilities were 1384 uv. In the sesame oil group the figures are 4100 uv. for the first month and 2280 uv. for the last month, and for the methylcolanthrene group the variability in the first month was 2608 uv. and in the last month

2235 uv. These figures suggest a decrease in variability during the experiment in the control groups. A marked increase in variability resulting from an injection of sesame oil alone was noted, a variability which decreased during the experiment, but never reached the level of the control group. The induced-tumor group maintained approximately the same degree of variability throughout the entire experiment. This suggests that the sesame oil alone, because of a possible local tissue reaction, produces a good deal of variation, the effects of which are less than those obtained by methylcolanthrene dissolved in sesame oil. However, the methylcolanthrene-injected mice maintained the initial variability throughout the period of observation.

With the help of Professor Frank Shuttleworth of the Institute of Human Relations, an analysis was made of the data which appears in Table I. The same material is presented in graphic form in Figure 1. The examination of these data show a number of results to which attention may be called. Within a week following the injection of sesame oil alone, the means of the axial potentials arose from 2100 uv. to 2700 uv. During this same period the axial potentials of the animals receiving methylcolanthrene in sesame oil changed from 2050 uv. to 1950 uv., an insignificant variation. In the animals receiving sesame oil alone, the mean of all the axial potentials taken after recovery from the injection was 1578 uv. In the group receiving methylcolanthrene dissolved in sesame oil, as well as sesame oil, the means of the potentials between the xyphoid

TABLE I

	N	M	PE_M	SD	PE_{SD}
Experimental Group. Axial Potentials (Xyphoid positive)					
One obs. before injection	20	2050	581	3853	411
Two obs. after injection	40	1950	437	4092	309
Intermediate observations	116	1320	131	2097	93
Two obs. before cancer	32	1125	240	2012	170
Two obs. after cancer	31	855	237	1959	168
Later observations	75	—205	195	2503	138
Experimental Group. Chest Potentials (Left chest positive)					
One obs. before injection	20	—1250	378	2507	267
Two obs. after injection	38	26	381	3478	269
Intermediate observations	122	246	139	2274	98
Two obs. before cancer	34	—529	199	1721	141
Two obs. after cancer	33	76	265	2256	187
Later observations	70	—257	213	2637	150

Control Group. Axial Potentials

	N	M	PE_M	SD	PE_{SD}
One obs. before injection	10	2100	613	2871	433
Two obs. after injection	20	3700	520	3444	368
Later observations	90	1578	167	2348	118

Control Group. Chest Potentials

	N	M	PE_M	SD	PE_{SD}
One obs. before injection	10	1400	552	2587	390
Two obs. after injection	20	—750	498	3300	352
Later observations	90	—878	170	2393	120

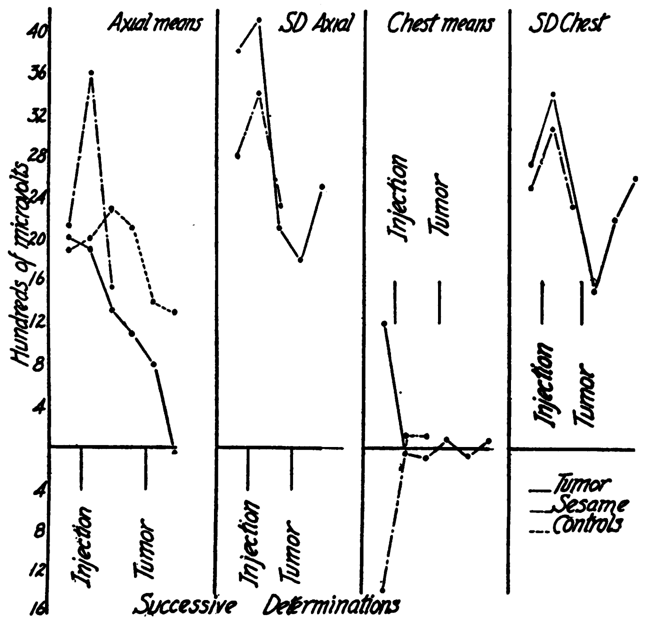
The decline in axial potentials of the experimental group from 2050 to —205 is 3.5 times its probable error. The increase in chest potentials of experimental group from —1250 to 246 is 3.7 times its probable error. Note the great contrast (2650) in chest potentials of experimental and control groups prior to injection. This is 3.7 times its probable error, whereas the true difference, of course, is zero.

Without exception the standard deviations increase after injection and then drop considerably below the initial average. Both declines in the experimental group exceed four times their probable errors.

and the symphysis after recovery from the injection declined from 1320 uv. to —205 uv. In all probability the injection of the carcinogenic agent started a process the net result of which was the gradual decrease in the potential difference between sternum and symphysis, a decrease which continued throughout the experiment. When sesame oil alone was injected, this process of decreased potential gradients did not occur. Likewise, in the control group, the means of the first set of readings was 1930 uv. and of the last set of readings, 1720 uv., a difference without significance.

Consideration of the chest potentials shows that in both the sesame oil group and in the carcinogenic group the potential gradient starting from a rather high point declined, following the injection, to a low point. Furthermore, the potentials across the chest remained relatively unaltered throughout the rest of the experiment. This is in rather striking contrast to the studies in spontaneous cancer in mice, in which the greatest effects seem to be in the chest potentials, whereas in this study the greatest effects seem to be in the axial potentials. A plot of the standard deviations of both axial and chest measurements is given in Figure 1 because they indicate the possible effect of injection and the appearance of cancer.

It seems reasonably clear from the data so far gathered, that a significant characteristic change in the electric pattern of these CBA mice occurs following the injection of methylcolanthrene in sesame oil, a change which continues up to the time of onset of induced tumors. All animals were autopsied and the tumors examined microscopically. Three types of sarcoma were obtained, a spindle-cell sarcoma, a round-cell sarcoma, and a sarcoma with many giant-cells. The number of mice showing each of the different types of



sarcoma was so few that it is impossible to determine whether or not there are valid correlates between type of tumor and the potential gradient.

Discussion

In the present paper a study was undertaken of the possible bio-electric disturbances induced within the organism by the presence of a known carcinogen. The carcinogen used was methylcolanthrene dissolved in sesame oil. Two sets of controls were employed, namely, sesame oil injection alone and normal mice of the same age and genetic strain (Strong, CBA) subjected to no treatment. In the course of the experiment 16 of the 20 mice injected with methylcolanthrene developed tumors—all of which were regarded as rapidly growing sarcomas varying somwhat in histological type. In those mice which eventually developed sarcoma there was a progressively decreasing reading of the axial potential gradient up to and through the presence of the induced tumor. This finding was not encountered in the controls nor in the nine receiving sesame oil alone. Contrasting with this, however, the mice receiving sesame oil alone showed a temporary enhancement of the axial potential readings during the first few weeks following the injection. Mice receiving methylcolanthrene dissolved in sesame oil did not show this increased axial gradient. This suggests the possibility, therefore, that the presence of methylcolanthrene in oil may interfere with the disturbance produced by sesame oil alone.

The presence of induced sarcoma in these mice apparently did not have any effect on the weekly variability of the axial potential gradient. The weekly variability for those mice having sesame oil alone was 2280 uv., whereas those mice which had induced sarcoma at the same time showed practically the same variability, 2235 uv. The controls receiving no treatment, however, had a weekly variability at the same time and age of 1384 uv., which is somewhat lower.

In the preceding paper dealing with the potential patterns of mice developing spontaneous carcinomas of the mammary gland the only probable deviation from the controls was the reading across the chest, whereas in the present investigation with a carcinogen, the only apparent deviation from the control was in the axial (xyphoid-symphysis) gradient. This peculiarity may be due to intrinsic differences in the genetic strain of mice used.

From the data of Marshall and Meader,[2] it seems that there is a great deal of individual variability, even in normal mice, from hour-to-hour and day-to-day measurements. This appears to be more pronounced in CBA than in C_3H mice. It is probably true, therefore, that certain disturbing elements not now under control, have a significant influence on potential gradient readings in general. Such being the case, it appears wise to conclude that the present study, interesting as it is from the standpoint of the intrinsic changes going on in the course of the induction of tumors, should not be taken as a specific diagnostic response caused by the onset and presence of a chemically induced neoplasm.

References

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