TECHNICAL PAPERS

Adrenal Changes in Animals Bearing Transplanted Tumors

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The purpose of this preliminary note is to report the changes observed in the adrenal and thymus glands of mice bearing sarcoma 180 tumors. This study was undertaken in view of the accumulating evidence suggesting adrenal dysfunction in animals (*1, 2, 4, 8*) and in patients (*5, 6, 9*) with neoplastic growths.

For the purpose of the experiment, young adult, female mice of the Carworth Farm CFW strain were chosen. In half of these, two 5-mg pieces of fresh sarcoma 180 tissue were implanted by trocar in the axillary regions; the remaining animals served as controls. The mice were kept under normal laboratory conditions, receiving Purina Laboratory Chow and tap water. Twenty-four hours prior to sacrificing, suitable numbers of animals were removed from both groups and placed in a constant-temperature room (25 ± 1° C), without food but with water to drink. On the 6th, 9th, and 12th days following implantation, the animals were killed by decapitation and the tumor tissue, thymus, and both adrenals dissected and weighed. The adrenals, having been dissected free from fat and decapsulated prior to weighing, were ground in 2% metaphosphoric acid solution and assayed colorimetrically (*3*) for their ascorbic acid content. The organ weights (expressed as mg/100 gm of body weight) and ascorbic acid levels, together with the standard errors, are given in Table 1. The values for the adrenals refer to both glands.

The values listed in Table 1 indicate that with increasing tumor size there is a progressive adrenal hypertrophy and thymus involution, the latter apparently reaching a minimum at the 9th day following implantation. A significant lowering of the ascorbic acid of the adrenal becomes apparent on the 9th day and continues to drop on the 12th day, evidently tending toward a lower level which would coincide with the death of the animals on or about the 15th day (*11*). The excessively high adrenal weights in the 12-day control group are probably due to coincidence with a change in the estrus cycle of the mice (see *12*), since these values were not apparent in the results of a second experiment listed in the lower part of the table.

TABLE 1

BODY AND ORGAN WEIGHTS OF MICE BEARING SARCOMA 180 TUMORS
(Averages and Standard Errors)

Duration (days)	No. of animals	Final body wt.* (gm)	Final tumor wt. (mg)	Thymus (mg/100 gm of b.w.)	Adrenals (mg/100 gm of b.w.)	Adrenal ascorbic acid (mg/100 gm of tissue)
6	Controls 12	20.6 ± 0.5	.....	213 ± 17.7	24.7 ± 1.9	283.3 ± 10.5
	Treated 12	21.2 ± 0.5	251 ± 34	174 ± 18.3	28.0 ± 1.7	273.0 ± 13.0
9	Controls 12	20.5 ± 0.6	.....	212 ± 12.8	25.8 ± 1.4	245.0 ± 9.9
	Treated 12	19.3 ± 0.4	445 ± 22	133 ± 11.2	32.2 ± 1.2	181.0 ± 14.9
12	Controls 12	20.1 ± 0.6	.....	211 ± 12.1	30.1 ± 1.7	270.0 ± 3.3
	Treated 12	17.9 ± 0.6	854 ± 96	140 ± 29.4	32.2 ± 3.3	174.5 ± 4.7
12	Controls 10	20.3 ± 0.7	.....	176 ± 13.1	26.5 ± 2.0	281.6 ± 15.9
	Treated 10	18.3 ± 0.7	.....	92 ± 24.0	34.1 ± 1.7	158.0 ± 8.7

* Includes weight of tumor tissue.

An immediate interpretation of the above findings would be that the tumor behaves as a nonspecific stimulus inducing the changes associated with the "general adaptation syndrome" described by Selye (*10*). In this syndrome, stimulation of the adrenals occurs by way of the pituitary and is indicated by their hypertrophy (*12*) and lowered cholesterol and ascorbic acid levels (*7*) and by involution of the thymus (*10*). The findings reported here would, if interpreted in this way, coincide with the terminal or exhaustion phase of the adaptation syndrome. Comparable observations have been made by other investigators in rats bearing Walker carcinoma 256 (*1, 2*); the fact that the adrenal hypertrophy could not be induced in hypophysectomized rats (*1*) lends support to this interpretation. However, from the evidence presented here, one cannot dismiss the possibility that the

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sarcoma 180 tumor has a direct effect on the adrenal or the thymus glands or both. Studies are now in progress which will determine this point.

It must be emphasized that results obtained with transplanted tumors (particularly such atypical growths as sarcoma 180) should be interpreted with reserve. Only when similar observations are made in an inbred strain of animals bearing a spontaneous form of cancer should any general conclusions be made regarding the adrenal changes and their relation to the natural disease.

References

1. Ball, H. A., and Samuels, L. T. *Proc. Soc. exp. Biol. Med.*, 1938, **38**, 441–443.
2. Bloor, W. R., Haven, F. L., and Randall, C. Proceedings of the American Association for Cancer Research, 39th Annual Meeting, Atlantic City, March 1948.
3. Carruthers, C. *Ind. eng. Chem.* (Anal. ed.), 1942, **14**, 826–828.
4. Dalton, A. J., and Peters, V. P. *Nat. Cancer Inst. J.*, 1944, **5**, 99–109.
5. Dobriner, K. Abstracts of Cancer Research Congress, St. Louis, 1947, 24.
6. Dobriner, K., Lieberman, S., Hariton, L., Sarett, L. H., and Rhoads, C. P. *J. biol. Chem.*, 1947, **169**, 221–222.
7. Long, C. N. H. *Recent progress in hormone research.* (Vol. 1.) New York: Academic Press, 1947. Pp. 99–122.
8. Murphy, J. B., and Sturm, E. *Cancer Res.*, 1947, **7**, 417–420.
9. Reifenstein, E. C., Jr., Homburger, F., and Dobriner, K. (To be published.)
10. Selye, H. *J. clin. Endocrinol.*, 1946, **6**, 117–230.
11. Sugiura, K. Private communication.
12. Tepperman, J., Engel, F. L., and Long, C. N. H. *Endocrinology*, 1943, **32**, 373–402

Use of Streptomycin-dependent Strains of Bacteria for Demonstrating the Ability of Microorganisms to Produce Streptomycin[1]

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Attention was previously directed (*6*) to the fact that streptomycin-producing strains of *Streptomyces griseus* can be identified by their sensitivity to a specific actinophage. Since this actinophage does not act upon other actinomycetes, however, the method could not be used to demonstrate streptomycin production by organisms other than *S. griseus*. For this purpose another method is suggested. It is based on the ability of certain bacteria, grown in media containing large amounts of streptomycin, to give rise to a mutant which is able to grow only in the presence of streptomycin in the medium.

The production by various bacteria of two types of mutants, one resistant to streptomycin and able to grow in ordinary media and another unable to grow in media free from streptomycin, was first demonstrated by Miller and Bohnhoff (*2, 3*) and later confirmed by Kushnick, *et al.* (*1*) and Paine and Finland (*4*). The latter spoke of the first type of variant as "resistant" and of the second as "dependent," to distinguish the two from the original, or "sensitive," culture. These strains may be designated as *rs, ds,* and *ss,* respectively.

In a comprehensive study on the distribution of resistant and dependent cells of *Escherichia coli* in a broth culture (incubated for 28 hrs at 28° C) of this organism, it was found that one resistant and one dependent cell were present among 1,500,000,000 normal sensitive cells. This was determined by plating the normal culture upon an agar medium containing 15 μg/ml of streptomycin. The *E. coli ds* grown on nutrient agar or in broth containing streptomycin produced the typical gram-negative rods; these were somewhat thinner and slightly longer than the cells of *E. coli ss,* the sensitive mother culture, from which the *E. coli ds* was isolated. When transferred to media free from streptomycin, the *E. coli ds* cells failed to divide, although they increased enormously in length. Streptomycin appears to act for this culture as a growth factor essential for cell division rather than as a substrate or a nutrient, since no destruction of streptomycin takes place.

The growth of *E. coli ds* in ordinary streptomycin-containing broth could be measured by making turbidimetric readings, using a Cenco Sheard-Sanford Photelometer. When the influence of streptomycin concentration in the medium upon the growth of this organism was measured, a definite correlation was obtained, up to a certain point, between the concentration of the antibiotic and the growth, as measured by turbidity, of the culture. This is brought out in Table 1. Frequently the control or streptomycin-free broth, especially when heavily inoculated, showed a certain increase in turbidity; this is primarily, at least at first, a result of the increase in the size of the cells in the inoculum rather than of the actual multiplication of the cells.

TABLE 1

Relation Between Concentration of Streptomycin and Growth of Streptomycin-dependent Strain of *E. coli*

Concentration of streptomycin in broth (μg/ml)	Incubation (hrs)			
	10	24	51	73
	Turbidimetric readings*			
0	0	0	0	0
1	0	2	9	9
5	1	6	12	12
10	2	10	14	14
20	4	13	17	19
30	5	14	20	23
40	6	15	22	26
50	6	15	21	25
100	7	16	21	24

* One ml of a suspension of an 18-hr-old streptomycin-dependent culture (*E. coli ds*) containing 37×10^5 viable cells was used as the inoculum.

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