litic virus, just as does the cationic form, with the advantages of cerebral penetration and comparatively low toxicity, but a more soluble compound than the bismuthate would be desirable.

4829

A Demonstration of the Curability of Malignancy in Rats by a Low Pressure Environment.

E. S. SUNDSTROEM AND G. GIRAGOSSINTZ.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Previous workers[1] who have carried out experiments on the effect of lowered oxygen tension on malignant neoplasms have reported a retarded growth and an extensive necrosis but no positive cures. They evidently missed the time when the factors which ultimately may annihilate the cancer reach their highest potency. Our success is attributable to (1) an adequate period of exposure, (2) a gradual adaptation to successive steps of decreasing oxygen tension, and (3) an uninterrupted maintenance of the final pressure throughout the experiment. We are not prepared to emphasize the superiority of our method, the reduction of the total pressure, as compared with the adjustment of the oxygen tension at normal atmospheric pressure. We believe, however, that it is technically more convenient and that it affords a greater margin of safety.

Our low pressure chambers are improved models of the apparatus described previously by one of us.[2] The pressure is controlled automatically and is disturbed neither by any manipulation in the care of the animals nor by the removal and the replacement of individual rats. A series may therefore be continued for almost an unlimited time, although the maximal exposure time in our cancer work has been 6 weeks exclusive of the one week adaptation period. Our results for the first 2 weeks have closely corresponded to those of other investigators and we shall, therefore, in this report consider mainly those series which have lasted longer.

We have employed 2 pressure levels: 300 and 360 mm., corresponding in altitude to 25,000 and 20,000 feet respectively. With

[1] Warburg, O., *et al.*, *Klin. Wochenschr.*, 1926, v, 829. Campbell, J. A., and Cramer, W., *Lancet*, 1928, ccxiv, 828.

[2] Sundstroem, E. S., and Bloor, W. R., *J. Biol. Chem.*, 1920, xlv, 155.

a few exceptions, adapted rats continue to do quite well in both of these environments and we believe that even the lower pressure level would also be endurable to patients. When the gradual adaptation time is omitted, on the other hand, one half of the rats succumb at the 300 mm. level. It appears from our statistics for adapted cancer rats that the mortality has been relatively high but these figures include a number of preventable deaths. The majority of the deaths have been due to accidents, for instance, irregularity in the ventilation, or to unrecognized ailments of the lungs, or to the choice of unsuitably large tumors for the treatment. Such tumors disintegrate very rapidly, rupture and become infected. The deaths of these rats have probably been due to the absorption of noxious material. Very few of such cases have been completely cured.

The experiments have been carried out on the Flexner-Jobling carcinoma and the Jensen sarcoma. We have observed a few spontaneous retrogressions occurring in very small tumors of the former type but never when they had reached the size we employed in our earlier low pressure work. On the other hand, we have noted 2 retrogressions in our sarcoma controls with respect to tumors which were equally large (15-30 mm. in diameter) as those we have treated in our later work. We were informed by Dr. C. F. Cori of the Cancer Institute in Buffalo, who was kind enough to supply us with breeders (and also with the sarcoma strain*) that retrogressions are very infrequent in this strain of rat.

We have inoculated successfully nearly 300 rats of the aforementioned strains of neoplasms. After deducting the numbers of such cancerous rats which we have used as controls, and of those which were treated for shorter periods or used for special experiments, we have tabulated the results for the remaining 133 rats which were given a treatment of from 3 to 6 weeks. The data are summarized in Table I. The term "definitely cured" is used for cases in which the animals were observed for periods of 2 to 6 months after their removal from the tanks. The tumors were considered to have been "apparently cured" in cases in which the crucial test of extended observation was not or could not be applied, for instance, with respect to dead rats, but in which the histological examination indicated a complete necrosis of the neoplasm.

In addition to the experiments involving low pressure at ordinary room temperature we have also considered the possibility that a raised temperature (usually 35° C.) could act as an adjuvant to the

* We are indebted to Dr. F. C. Wood at the Cancer Institute of Columbia University for the carcinoma strain.

low pressure in annihilating malignancy. It appears that the histological picture of tumors in this latter series is altered in such a way so as to suggest that the combined environmental method of cancer treatment may prove advantageous. The "tropical mountain climate" appears to lessen the mortality of the rats. Since it is yet doubtful whether the combined treatment affects the percentage of cures we have not entered any subdivisions based on temperature in this report.

TABLE I.

Type of Tumor	Pressure	Number of Rats	Mortality	Definite Cures	Apparent Additional Cures
	mm.		%	%	%
Carcinoma	300	32	28	34	22
	360	33	21	24	12
Sarcoma	300	29	14	83	14
	360	39	38	38	13

These data indicate a correlation between the destructibility of the malignant growth and the pressure level used and, on the other hand, a greater vulnerability of the sarcoma strain. We believe, however, that the seemingly speedier response of the latter strain as compared with the carcinoma may be partially due to the inequality in the size of the tumors which we used for the 2 series. The results of the "sarcoma, 300 mm." series are, furthermore, almost too good to be reproduceable. It is probable that in this series some unknown factor, for instance of seasonal origin, may have assisted the low pressure in annihilating the cancer.

Ten rats were inoculated with sarcoma a few minutes before they were placed in the tank. Contrary to our expectation that the tumor fragments would fail to grow we obtained 100% takes. The tumors underwent the same phases of growth and subsequent necrosis as tumors which were already large at the start. Such results are difficult to interpret under the assumption that the reduced oxygen tension is only directly responsible for the destruction of cancer in low pressure environments. One is tempted to surmise that the reduction of the oxygen tension may also act in an indirect way, for instance, by effecting a diminution of some substance which is essential for the growth and maintenance of the cancer or an accumulation of some substance, known or unknown, which is destructive to the tumor. In our studies of the time relations in the levels of certain blood constituents, for instance with respect to the sugar and non-protein nitrogen, we have obtained results which suggest a parallelism with the time relations in the effectivity of the low pressure treatment on cancer.

One would expect no lasting effect of the oxygen deficiency if its action were only direct. On the other hand, if it produces some biochemical modifications which are injurious to malignant growths, these modifications might affect also a cancer which is inoculated subsequent to the cessation of the low pressure exposure. One of our more recent experiments seems to prove that malignancy acquired by pretreated rats may run a different course than in untreated animals.

Eleven normal rats were exposed to a 300 mm. pressure and inoculated with sarcoma on the first or second day after their removal from the tank. The control series comprised 9 rats which were inoculated at the same time with fragments from the same sarcoma. The takes were in both series 100%. The tumors grew at first at an equally rapid rate. Later 3 of the pretreated neoplasms retrogressed completely, as compared with one retrogression among the controls. The other control tumors grew to a huge size and presented an almost solid mass of normal sarcoma tissue. On the other hand, the tumors of the pretreated animals became very soft and the section of them showed that they had liquified, leaving only a thin rim of cancer tissue of doubtful normality.

4830

A Comparison of Ultrafilterable Serum Calcium and Cerebrospinal Fluid Calcium in Humans.*

DAVID M. GREENBERG. With the Assistance of H. E. Ballard and J. B. Dalton.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Cameron and Moorhouse[1] have proposed using the cerebrospinal fluid calcium as a measure of the diffusible calcium of the blood plasma, arguing that the choroid plexus as a living, colloid impermeable membrane gives us a more perfect distribution of the diffusible constituents of the blood than can be obtained in any *in vitro* manner. This implies both that the cerebrospinal fluid is formed by a process of diffusion rather than of active secretion and also that the fluid is continuously in diffusion equilibrium with the constit-

* Presented at the Ann Arbor meeting of the Society of Biological Chemists, April, 1928.

[1] Cameron, A. T., and Moorhouse, V. H. K., *J. Biol. Chem.*, 1925, lxiii, 687.