PROPERTIES OF THE CAUSATIVE AGENT OF A CHICKEN TUMOR

II. THE INACTIVATION OF THE TUMOR-PRODUCING AGENT BY MONOCHROMATIC ULTRA-VIOLET LIGHT*

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Early work on Chicken Tumor I indicated that, while the sarcoma cells are as susceptible to ultra-violet radiation as other cells, the etiological agent separable from the cells is relatively resistant (1). Later Baker and Peacock (2) estimated that five times the lethal dose for pathogenic bacteria was not sufficient to destroy the activity of the chicken tumor agent, and that even eight times the amount did not invariably destroy the activity (7). This observation was confirmed in general by Illingworth and Alexander (3). All these observations were made without particular reference to wave length or the absolute energy involved. One of the present authors (Gates (4)), using measured monochromatic light and a standard technique, has been engaged in a comparative study of the energies required to kill or inactivate various organisms or biological agents at single wave lengths in the ultra-violet region. By plotting the energies required against the corresponding wave lengths, similar graphs are obtained for comparing the qualitative and quantitative action of ultra-violet light. This method offers an opportunity to compare the reaction of the tumor agent with that of bacterial cells, virus or phage.[1]

[1] A preliminary report on this work was published in the *Internat. Conf. Cancer*, London, 1928, 33.

Method

1 gm. of finely mashed Chicken Tumor I, or an equal amount of tumor desiccate, was emulsified with 10 cc. of water, thoroughly shaken, centrifuged at high speed, and the supernatant fluid passed through a filter paper. The bottom surface of a small Petri plate (4.5 cm. in diameter) was covered with enough melted agar to form a layer 1.5 to 2 mm. thick after solidification.[2] On this smooth surface 2 drops of the thick tumor filtrate was evenly spread and allowed to stand at room temperature for about 45 minutes, when sufficient drying had occurred to prevent flowing of the material. Uniform strips, 3 x 20 mm., were then cut from the middle of the agar plate and exposed to varying doses of ultra-violet radiation. With a quartz mercury arc as the source of energy, the specimens were placed behind the exit slit of a large monochromatic illuminator for various intervals of time. The wave lengths selected lay between λ 238 and λ313 mμ, and the intensity of the radiation at each wave length (measured in ergs per mm.[2] sec. by means of a standardized thermopile and high sensitivity galvanometer) multiplied by the time of exposure gave the total energy per mm.[2] for each exposure. Immediately afterwards the strips were loaded in 16 gauge lumbar puncture needles and injected intradermally in chickens, each chicken receiving also a control unexposed strip which had been kept under the same general conditions as the test specimens.

The uniformity of the control "takes" and the reasonable regularity of results with the exposed specimens indicated that the presence of the neutral agar had no significant effect on the reactions.

RESULTS

The results of 624 irradiation tests are shown graphically in Text-fig. 1. The curve is based on the points at which the agent was attenuated to such an extent that tumors resulted from less than 50 per cent of the test inoculations. The results for λ313 mμ are not shown, as the tumor agent was not inactivated even by an exposure of 80,000 ergs per mm.[2]

Ultra-violet inactivation curves for a bacterium (4), a typical virus (5) and a phage (6) have been plotted in Text-fig. 1 for comparison.

The energy required at each wave length to inactivate the tumor agent is far greater than that required to kill or inactivate bacteria, virus or phage.[3] It is of equal importance to note the relative differ-

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[2] In preliminary experiments carried out to test the suitability of this method, gelatine was used, but as it was found that this substance had a definite enhancing action on the tumor agent, agar was substituted.

[3] Baker and Nanavutty, working with an unresolved ultra-violet spectrum, estimate that phage has the same degree of susceptibility as bacteria, that the chicken
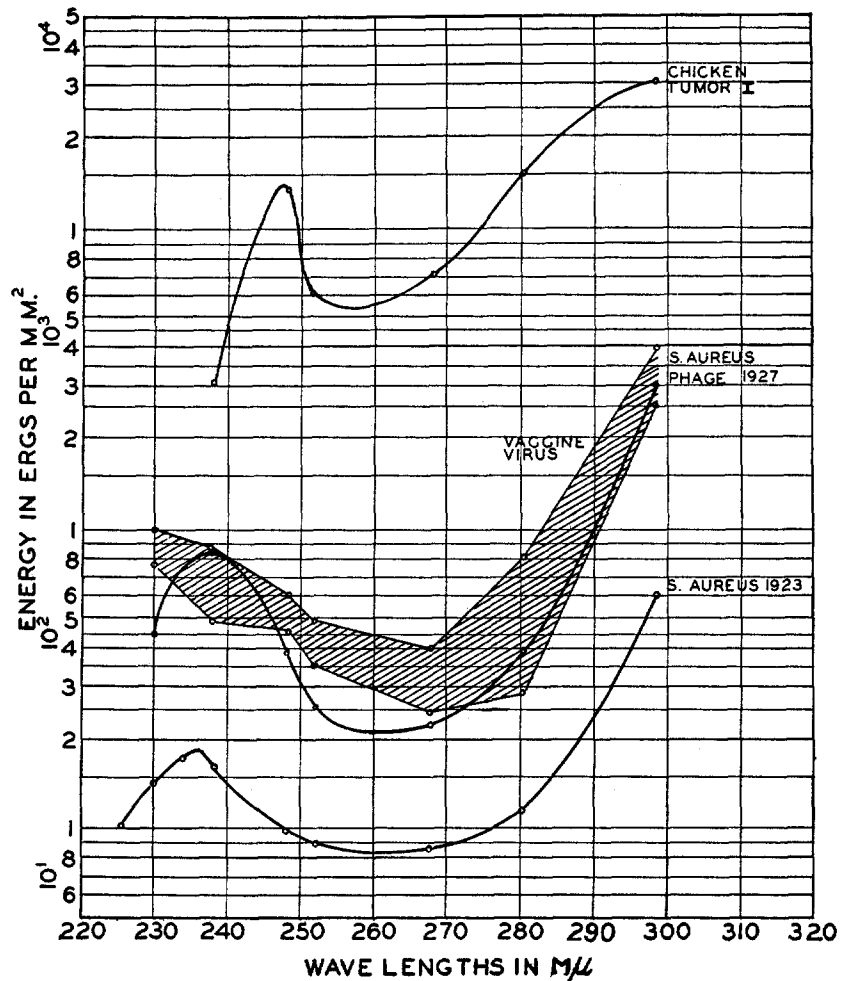
TEXT-FIG. 1. The points on the curves drawn for *S. aureus*, *S. aureus* phage and Chicken Tumor I represent the energies required to reduce the subsequent colony or plaque formation or tumor takes to 50 per cent of those obtained with control specimens.

The cross-hatched area shows the limits of energy in various experiments which resulted in the failure of exposed vaccine virus to produce lesions in susceptible rabbits.

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tumor is 8 times more resistant, while ferments and antibodies are 20 to 120 times more resistant (7).

ences in activity of the various wave lengths examined. The general form of the curves for bacteria, virus and phage is similar. Contrasting these with the curve for the tumor agent, it is seen that among the shorter wave lengths tested the most active for the tumor agent (λ238) is least active for the other group; and the least active for the tumor agent (λ 248) is in the range of the most active wave lengths for bacteria, virus and phage.

DISCUSSION AND SUMMARY

Even though part of the energy of the incident light is probably absorbed by chemical entities which play no part in the specific reaction of inactivation, nevertheless the wave lengths most active in destroying biological cells or agents will presumably be found to be among those absorbed in the highest proportion. This would indicate that the curves here presented are approximately reciprocal to the coefficients of absorption of particular substances, the destruction of which caused the inactivation of the agents or the death of the cells. The similarity between the curves for bacteria, virus, and phage, both in shape and in total involved energies, suggests the presence of a common factor, or of closely related chemical entities, sensitive to ultra-violet light, whereas the data for the tumor agent suggest that its inactivation is due to the destruction of a substance having an essentially different spectral absorption, and therefore of a different chemical character. While the amount of ultra-violet energy required to affect the tumor agent is great, it is still less than that involved in the inactivation of some of the enzymes (7).

A study is under way to compare the deduced spectral analysis with the actual coefficients of absorption of the highly purified tumor agent.

BIBLIOGRAPHY

1. Rous, P., J. Exp. Med., 1913, 18, 416.
2. Baker, S. L., and Peacock, P. R., Brit. J. Exp. Path., 1926, 7, 310.
3. Illingworth, C. L. W., and Alexander, G. L., J. Path and Bact., 1927, 30, 365.
4. Gates, F. L., J. Gen. Physiol., 1929, 13, 231, 249; 1930, 14, 31.
5. Rivers, T. M., and Gates, F. L., J. Exp. Med., 1928, 47, 45.
6. From an unpublished study by Muckenfuss, R., and Gates F. L.
7. Baker, S. L., and Nanavutty, S. H., Brit. J. Exp. Path., 1929, 10, 45.