

CCLIX. THE ACTION OF ASCORBIC ACID ON TUMOUR METABOLISM.

A PRELIMINARY NOTE ON THE EFFECTS OBSERVED FOLLOWING INJECTIONS INTO MICE WITH TAR-INDUCED NEOPLASMS.

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IN view of the recent syntheses and extended knowledge of the chemical and biological properties of ascorbic acid (vitamin C) it was believed that the following brief account of experiments upon tar painted mice receiving injections of the synthetic ascorbic acid would be of interest to workers in cancer and also in other fields of biochemical research. The experiments were suggested by the communication of Jorrissen and Belinfantes [1933] who found that lactic acid was oxidised *in vitro* by vitamin C and put forward the idea that the presence of ascorbic acid in the tissues might depress the amount of lactic acid accumulating in the tumours with resultant inhibition of the invasion of the surrounding areas by the malignant process. Mellanby [1934] had found that in animals fed with diets rich in ascorbic acid given in form of lemon juice, such treatment slightly inhibited the growth of some rat and mouse tumours. It might, however, be suggested that other physiologically active principles would be administered by this particular technique so that in the tests described below only pure synthetic ascorbic acid was utilised and given subcutaneously.

It appears to have been satisfactorily demonstrated that lactic acid is produced in considerable quantities during the metabolic activity of tumours and probably more abundantly by epithelial than by connective tissue growths and especially by highly malignant types of neoplasms. Tar epitheliomas produced in white mice have been found by many workers to be very resistant to therapeutic effects and invariably to be highly malignant in nature, so that these should prove a suitable type of growth for observing the effects of such injections.

These have been studied in three series of animals, whilst suitable numbers of control mice have been maintained under similar conditions of accommodation, feeding *etc.* Series "A" was comprised of 25 mice painted twice weekly with a carcinogenic coal tar and injected subcutaneously at similar intervals with 0.2 ml. of 0.5 % ascorbic acid in saline, the solution being prepared immediately before use. Series "B" consisted of 20 mice which had been painted for a period of about 18 weeks and upon which small warts, visible to the naked eye, had been apparent for 1-3 weeks. In this case injections were commenced at "wart stage" and tar applications were continued as with the other series. The third series "C" was constituted of mice bearing tar tumours which were judged to be

definitely malignant epitheliomas at an early stage of development. For each series at least an equal number of control animals without injections were observed, while other series of animals receiving various pharmaceutical preparations have also been available for comparison. All the animals were drawn from a stock which had been inbred for several years, and which has been used for a large number of comparative experiments in the laboratory so that it would be unlikely that marked differences in reaction would be attributable to genetic variations in the test material.

The results up to the present appear to be unmistakable and somewhat remarkable. In the series "A" the warts appeared at much the same period as in the control set, approx. 18 weeks. The animals in the "B" series, however, reacted in a definite way for the warts quickly developed to the malignant type, invaded a large area of the back, soon ulcerated and killed the animals. The average time for this was only about half that for the controls to progress to a similar degree. The observations were confirmed by the "C" series in which the rapidity of the invasion of surrounding skin and tissues was such that the growths after a further 2 or 3 weeks injections assumed very large dimensions, often 3-4 cm. in diameter.

In several instances metastases have been found in the lungs though the work is not advanced sufficiently for comparison of the frequency of such deposits. The experiments are being continued with larger numbers of animals to obtain fuller details, and variations in technique are to be introduced. Hence only general descriptions are made in this communication. In the meantime it would no doubt be of interest to other workers to examine the effects of such treatment on different stocks and on other tumours, since the effect, apparently, is chiefly in the cancerous phase. That ascorbic acid passes into the blood and other tissues is demonstrated by the fact that Demole [1934] was able to titrate the vitamin in the urine of dogs following subcutaneous injections of ascorbic acid and also found that such injections caused the figures for reducing substances in the blood to rise to values of 140-160 mg./100 ml. decreasing in 2 hours to 110-120 mg./100 ml.

The interpretation of the results is difficult, especially on account of contradictory evidence briefly summarised here. The "defect" in malignant cells has been ascribed to a deficiency in lactic dehydrogenase. Thus, since carcinogenic hydrocarbons inhibit the oxidation of lactic acid by muscle and yeast dehydrogenase [Boyland, 1932; 1933, 1], when such hydrocarbons are applied to the organism local accumulation of lactic acid may ensue. The oxidation products of dibenzanthracene, although not actively carcinogenic, were also found by Boyland and Boyland [1934] to inhibit the respiration and glycolysis of normal and tumour tissue. Tumour cells apparently survive and grow in media containing lactic acid in excess of physiological limits and are less affected than normal cells. Conditions under which lactic acid accumulates may thus be favourable to the multiplication of malignant cells.

Boyland [1933, 2] found that a reducing substance with some properties in common with ascorbic acid occurred in a variety of tumours; it was subsequently discovered however that the constituent was not ascorbic acid [Harris, 1933].

It has also been suggested [Birch and Dann, 1933] that vitamin C may be linked with glutathione in one oxidation-reduction system for they found that in a number of organs the content of vitamin C ran parallel with that of the glutathione.

In any case ascorbic acid is a powerful reducing agent, and an explanation

of its stimulating effect on the proliferation of the tumours may be that it interferes with the action of the dehydrogenase of the tissues, thereby causing lactic acid to accumulate and thus inducing tumour extension.

On the other hand the work of Parfentjev and his co-workers is of some importance as it is apparently contrary to this suggestion. These workers concluded that sodium lactate inhibits multiplication of cells [1931] and also observed the effect of injections of sodium lactate combinations on sarcomatous rats with the result that, in many instances, transplants of sarcoma completely disappeared [Parfentjev *et al.*, 1932]. They had previously satisfied themselves that such injections increased the lactic acid content of the blood [Parfentjev, 1931].

The effects noticed might, of course, be ascribed to such a factor as local or systematic change in p_{H} rather than the result of enzyme derangement. The results, however, may indicate that the rôle of ascorbic acid in accelerating tumour growth consists in the oxidation of lactic acid, thereby causing the removal of an inhibitory metabolic product and thus facilitating tumour extension.

The results have been discussed in this paper from the point of view that lactic acid is a significant factor in tumour growth. In conclusion, however, it is felt that the observations justify the comment of Copisarow [1934], who pointed out that lactic acid is only one of the intermediary products of abnormal glycolysis and is neither specific in, nor experimentally productive of, cancer, while uncontrolled disturbance of the delicately balanced hormone equilibrium in the system either by overdosage or overproduction would be fraught with danger.

SUMMARY.

The injection of ascorbic acid into white mice did not alter the induction period for the production of tar warts.

Under its influence, however, rapid development to the malignant stage and invasion of the surrounding tissue with accumulation of large necrotic masses was observed.

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REFERENCES.

- Boyland (1932). *Nature*, **130**, 274.
- (1933, 1). *Biochem. J.* **27**, 802.
- (1933, 2). *Biochem. J.* **27**, 786.
- and Boyland (1934). *Biochem. J.* **28**, 285.
- Birch and Dann (1933). *Nature*, **131**, 469.
- Copisarow (1934). *Chem. Ind.* **53**, 135.
- Demole (1934). *Biochem. J.* **28**, 770.
- Harris (1933). *Biochem. J.* **27**, 805.
- Jorissen and Belinfantes (1933). *Chem. Ind.* **52**, 896.
- Mellanby (1934). *Ann. Rep. British Empire Cancer Campaign*, p. 83.
- Parfentjev (1931). *J. Biol. Chem.* **93**, 797.
- Devrient and Sokaloff (1931). *Proc. Soc. Exp. Biol. Med.* **28**, 536, 538.
- — Suntzoff and Sokaloff (1932). *Amer. J. Cancer*, **16**, 366.