

Effect of Amethopterin and 6-Mercaptopurine on the Pattern of Oxygen Tension Measurements in the Fluid Phase of Serum Agar Cultures of Bone Marrow Flecks

WE have recently described an electro-chemical method of measuring the oxygen tension in the fluid phase of serum agar cultures of bone marrow flecks¹. Oxygen tension decreased, at first fairly quickly and then more slowly over a period of three days. This was ascribed to the utilization of oxygen by the surviving cells. In this communication we describe some preliminary experiments upon the effect of amethopterin and 6-mercaptopurine on the oxygen tension pattern of similar cultures. At the beginning of the experiments the culture fluid was equilibrated with air containing 5 per cent carbon dioxide; the carbon dioxide was required to keep the pH of the fluid in the physiological range. After a number of hours the fluid was replaced either with fresh medium alone or with medium containing the drugs. As this fluid was also equilibrated with the air-carbon dioxide mixture, the oxygen tension rose but not usually to the starting level. This may have been due to the mixing of the fresh medium with the relatively anoxic serum agar also present in the culture chamber. Thereafter, in the normal controls the oxygen tension continued to fall until the experiment was concluded. This is illustrated by the control curve seen in Fig. 1.

When a medium containing a 1 mM concentration of amethopterin was added to the culture of normal marrow there was a definite alteration in the pattern which is illustrated in Fig. 1. After a latent period of about 8 hr. the curve flattened; this we think is due to interference with the normal oxygen metabolism of the cells. It did not occur in 0.25 mM concentrations; 0.5 mM concentrations showed an intermediate effect.

The findings with 6-mercaptopurine were comparable. We found that a 6-mM concentration caused a flattening in the oxygen tension curve after a latent period of about 20 hr.; this did not occur with 2-mM concentrations and 4-mM concentrations had an intermediate effect.

The oxygen tension patterns of the fluid phase of serum agar cultures of marrow flecks from a case of acute leukaemia have also been studied. The patient, a woman aged forty, was admitted to hospital with pneumonia. The peripheral blood showed a sub-leukæmic picture, and the bone marrow was stuffed

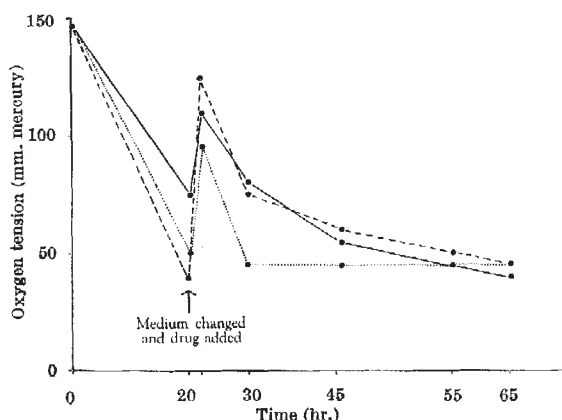


Fig. 1 ●—●, No drug control; ●—●—●, 1 mM amethopterin; ●—●—●, 0.25 mM amethopterin

with primitive leukæmic cells. After a stormy course and treatment with antibiotics, steroids and 6-mercaptopurine a remission was induced. This lasted 5 months, after which relapse and death occurred. At no time was the patient given antifolic drugs. At the time of the cell culture the marrow showed predominantly primitive leukæmic cells. Four cultures were made without the addition of drugs, and the oxygen tension patterns resembled those found in normal marrow. 6-mercaptopurine was added to three cultures in concentrations of 6, 4 and 2 mM, respectively. The curve obtained with the culture containing the 6-mM concentration showed a levelling after about 10 hr.; those obtained from the 4- and 2-mM concentrations continued downwards. Amethopterin was added to three cultures in concentrations of 1, 0.5 and 0.25 mM respectively; in none of these was a levelling effect seen.

These preliminary results are of interest because they could form the basis of an *in vitro* test of the sensitivity of leukæmic marrow cells to various chemotherapeutic agents. This would be based on the hypothesis that the concentrations of drugs which would inhibit the oxygen uptake of sensitive cells might not affect cells which were resistant.

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¹ Davis, J. M. G., and Woodliff, H. J., *Blood* (in the press).

PLANT PHYSIOLOGY

Effect of Gibberellic Acid on Flower Differentiation in Petkus Winter Rye

It is known that application of gibberellic acid causes flowering of many biennials (*Hyoscyamus*, *Apium*, *Brassica*, *Daucus*, etc.)¹. In these cases gibberellic acid substitutes for cold (vernalization) treatment. Treatment of winter cereals (Petkus rye², barley³, winter wheat⁴) with gibberellic acid, on the contrary, has been found to have no effect on the induction of normal flowers. Chaylakhian⁵ has reported that treatment of non-vernalized oats, variety Victory, with gibberellic acid, under short-day conditions, caused formation of an increased number of developed panicles. This was not the case under long-day conditions. Paleg and Aspinall⁶ have reported that treatment of barley with gibberellic acid for several weeks immediately following emergence results in the formation of abnormal spikes.

The experiments reported here are concerned with the normal flower formation of Petkus winter rye by application of gibberellic acid. This cereal is, under the growing conditions of our experiments, a cold-requiring plant.

It has been found that, to be successful, treatment must occur at a particular stage in the development of the plant.

All the experiments were performed under the controlled greenhouse conditions of the Earhart Plant Research Laboratory. A description of this laboratory and the experimental facilities available in it has been given by Went⁷.

Plants of winter Petkus rye, grown under a constant temperature of 17° C., both in short-day (8 hr.) and long-day (16 hr.) regimes, were sprayed with