- Griffith, T. & Byerrum, R. H. (1959). *J. biol. Chem.* **234**, 762.
- Hamilton, P. B. (1958). *Analyt. Chem.* **30**, 914.
- Kearney, P. C. & Tolbert, N. E. (1961). *Plant Physiol.* **36** (Suppl.), xxvi.
- Kenton, R. H. & Mann, P. J. G. (1952). *Biochem. J.* **52**, 130.
- Kornberg, H. L. & Beevers, H. (1957). *Nature, Lond.*, **180**, 35.
- Kun, E., Dechary, J. M. & Pitot, H. C. (1954). *J. biol. Chem.* **210**, 269.
- Kun, E. & Hernandez, M. G. (1956). *J. biol. Chem.* **218**, 201.
- Kursanov, A. L., Kryakova, N. N. & Vyskrebentsera, E. I. (1953). *Biokhimiya*, **18**, 632.
- Link, G. K. K., Klein, R. M. & Barron, E. S. G. (1952). *J. exp. Bot.* **3**, 216.
- Maitre, V. & Dekker, E. E. (1961). *Biochim. biophys. Acta*, **51**, 418.
- Martin, S. M. (1955). *Chem. & Ind.*, p. 427.
- Michael, P. W. (1959). Ph.D. Thesis: University of Adelaide.
- Milhaud, G., Benson, A. A. & Calvin, M. (1956). *J. biol. Chem.* **218**, 599.
- Moore, S., Spackman, D. H. & Stein, W. H. (1958). *Analyt. Chem.* **30**, 1185.
- Moore, S. & Stein, W. H. (1954). *J. biol. Chem.* **211**, 907.
- Nakada, H. I. & Weinhouse, S. (1953). *J. biol. Chem.* **204**, 831.
- Richardson, K. E. & Tolbert, N. E. (1961a). *J. biol. Chem.* **236**, 1280.
- Richardson, K. E. & Tolbert, N. E. (1961b). *J. biol. Chem.* **236**, 1285.
- Schou, L., Benson, A. A., Bassham, J. A. & Calvin, M. (1950). *Physiol. Plant.* **3**, 487.
- Tolbert, N. E. & Cohen, M. (1953). *J. biol. Chem.* **204**, 649.
- Weinhouse, S. & Friedman, B. (1951). *J. biol. Chem.* **191**, 107.
- Zelitch, I. & Ochoa, S. (1953). *J. biol. Chem.* **201**, 707.

Biochem. J. (1963) **86**, 62

The Effect of Colchicine on the Acid-Soluble Ribonucleotides of Normal and Regenerating Rat Liver

By D. Y. WANG, A. L. GREENBAUM AND R. D. HARKNESS

Departments of Biochemistry and Physiology, University College London, Gower Street, London, W.C. 1

(Received 26 June 1962)

Although colchicine has long been known to act as a mitotic poison, the nature of its action is not understood. The results of Lettré & Albrecht (1951), showing that the effect of colchicine on mitosis in tissue culture could be reduced by the addition of adenosine triphosphate (ATP) to the medium, have drawn attention to the role of ATP in this process, and it has been suggested that it causes the mitotic spindle to contract by direct action on its constituents. Further, Benitez, Murray & Chargaff (1954) have shown that when ATP is added with colchicine to tissue cultures there is a decrease in the degree of mitotic arrest, although these workers found that the percentage of cells in the various phases of division in the presence of ATP was the same as that found in cultures to which no ATP had been added. The above evidence appeared to give ATP an important role in cell division and it seemed possible that, if the action of colchicine were primarily on the level of production of this compound, then the difference would manifest itself in the acid-soluble pool of ribonucleotides. For this reason measurements were made of the content of the various acid-soluble ribonucleotides in normal rat liver and in the livers of rats treated with colchicine. Further,

in order to exaggerate such a difference, if one existed, measurements were also made of the effect of colchicine on the acid-soluble ribonucleotides in the livers of partially hepatectomized rats at a time after the operation when the maximum rate of cell division would be expected to occur. Finally, a comparison was made between the effect of colchicine and Myleran, another mitotic poison, on the acid-soluble ribonucleotides.

METHODS

Animals. Adult male albino rats weighing about 200 g. were used. Those that were partially hepatectomized had the median and left-lateral lobes of the liver removed under ether anaesthesia as described by Higgins & Anderson (1931).

Colchicine. Colchicine, purchased from British Drug Houses Ltd., was dissolved in water (concn. 500 µg./ml.) immediately before use, and administered intraperitoneally in a dose of 1 mg./kg. body wt. It was given to partially hepatectomized animals 24 hr. after operation, i.e. at about the time when the maximum rate of mitosis in hepatic parenchymal cells would be expected.

Small portions of the livers of some of the partially hepatectomized animals were examined histologically; numerous arrested mitoses were seen in the animals treated

[illegible]

Thus at the shorter time-interval the main change is a general dephosphorylation resulting in an increase of the monophosphates at the expense of the triphosphates, whereas at the longer time-interval there appears to be a catabolic breakdown which results in the depletion in the triphosphates alone, an effect which is particularly marked with the 'ATP' peak.

Whether the dephosphorylation of UTP and GTP is secondary to a lowering of the ATP concentration or whether it is due to an increased enzymic dephosphorylation cannot be determined from the present results; nor is it possible to determine whether the dephosphorylation is a result or a cause of the mitotic inhibition. Nevertheless, the finding that treatment with colchicine leads to a very significant reduction in the concentration of ATP in the tissue is consistent with those schemes which envisage a role for ATP in mitosis and for the idea that the inhibitory action of colchicine on mitosis is effected by its action on the concentration of tissue ATP. In this connexion it is interesting to contrast the effect of Myleran on the liver acid-soluble nucleotides with that of colchicine. Although Myleran is a powerful inhibitor of mitotic activity it has almost no effect on the concentration of any of the individual acid-soluble ribonucleotides, and it would appear that this substance may well produce its inhibitory effect by a mechanism different from that of colchicine.

SUMMARY

1. The concentrations of the acid-soluble ribonucleotides in the livers of normal and partially hepatectomized rats and of normal and partially hepatectomized rats treated with either colchicine or Myleran have been measured.

2. Although the treatment with drugs had little effect on the majority of the nucleotides, colchicine caused the concentrations of NAD and AMP to increase and those of ATP, GTP and UTP to decrease.

We wish to acknowledge our debt to the Medical Research Council for a grant to one of us (D. Y. W.).

REFERENCES

- Benitez, H. H., Murray, M. R. & Chargaff, E. (1954). *Ann. N.Y. Acad. Sci.* **58**, 1288.
 Eichel, M. A. & Spirtes, H. J. (1954). *Arch. Biochem. Biophys.* **53**, 309.
 Gal, M. E. (1938). *Bull. Soc. chim. Biol., Paris*, **20**, 1188.
 Glock, G. E. & McLean, P. (1954). *Biochem. J.* **56**, 171.
 Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path.* **12**, 186.
 Holzer, H., Busch, D. & Kroger, H. (1958). *Hoppe-Seyl. Z.* **313**, 184.
 Hurlbert, R. B., Schmitz, H., Brumm, A. F. & Potter, V. R. (1954). *J. biol. Chem.* **209**, 23.
 Lettré, H. & Albrecht, M. (1951). *Naturwissenschaften*, **38**, 547.

Biochem. J. (1963) **86**, 64

Vitamins in Germination

DISTRIBUTION OF INOSITOL DURING THE GERMINATION OF THE DWARF BEAN, *PHASEOLUS VULGARIS*

By L. N. GIBBINS* AND F. W. NORRIS

Department of Biochemistry, University of Birmingham

(Received 18 May 1962)

Previous papers from these Laboratories discussed the determination of inositol in germinating oats (Darbre & Norris, 1956) and in the seed and young plants of the French bean (Darbre & Norris, 1957). Studies on inositol in germinating seeds have been described by Burkholder & McVeigh (1945*a, b*), Cheldelin & Lane (1943), Hinton (1942, 1947), Hinton, Peers & Shaw (1953) and Richardson & Axelrod (1957); but to date there is little information on the distribution of inositol in the dissected anatomical parts of the seed and young plant.

* Temporary address: N.R.C. Prairie Regional Laboratory, Saskatoon, Canada.

The present work is an extension of that described by Darbre & Norris (1957) and is a comprehensive survey of the distribution of total and free inositol in six anatomical parts of the germinating seed and seedlings of *Phaseolus vulgaris* at six morphologically distinct stages of growth taking place in total darkness.

EXPERIMENTAL

Materials

The seeds used were of the dwarf French bean, *Phaseolus vulgaris* var. Improved Canadian Wonder.