that the radioactive platelets have been damaged to some degree.

It is obvious that more investigation must be done before a final answer can be given but these experiments do indicate the sites where platelets may be destroyed and point up certain aspects of platelet metabolism heretofore not demonstrated. It is hoped in the future that this method may be applied to studies in human beings.

Summary. 1. Purified, unagglutinated platelet suspensions prepared from rabbit blood do incorporate radioactive phosphorus readily, and therefore lend themselves to life span studies *in vivo*. 2. These studies indicate that transfused rabbit radioactive platelets are rapidly removed from the peripheral blood and the radioactivity recovered in organs of the reticulo-endothelial system, namely spleen, liver, lung and bone marrow. 3. The problem of viability of these platelets is discussed. The evidence suggests that damage has occurred during manipulation. 4. These observations are important primarily in demonstrating aspects of metabolism and destruction of platelets.

The author wishes to thank Dr. Eugene Saenger of the Radioisotope Laboratory of the Cincinnati General Hospital for his valuable advice and assistance in obtaining and using the radioactive isotope.

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Received April 9, 1953. P.S.E.B.M., 1953, v83.

Reversible Inhibition of Cell Division and Enlargement in Plant Tissues by 2, 6-Diaminopurine.* (20418)

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Since the report by Hitchings and coworkers(1) that 2,6-diaminopurine (DAP) inhibits the growth of *Lactobacillus casei*, the compound has been used in many studies of growth in microorganisms and animals as well as in virus reproduction. In survey experiments, DeRopp(2) tested the chemical for action on growth of plant tumor tissue but observed no effects. In this paper are reported the results of experiments with several different tissues of higher plants in which DAP inhibits cell division—as it does in animals and microorganisms—and also inhibits cell enlargement and the formation of vegetative buds.

Cell division in tobacco stem segments. Sterile tobacco stem segments were obtained from 3 to 4 ft tall tobacco plants, Wisconsin No. 38, grown in a greenhouse. After removal of the leaves, the stems were cleansed by swabbing with 95% ethyl alcohol and then the outer layers down to the cambium were peeled off. The remaining cylinders were cut into sections 1 cm long. Three to 4 segments were obtained from each section by cutting it tangentially and the central core (pith) was discarded. These stem segments contained parts from the vascular-cambial region, xylem, internal phloem, and pith and averaged about 6 mm in width, 10 mm in length, and 2 mm (maximal) in thickness. They were placed pith side down on the surface of the basal or

* Supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council and in part by the Research Committee of the Graduate School on funds from the Wisconsin Alumni Research Foundation.

TABLE I. Inhibition of Budding and Callus Formation in Tobacco Stem Segments by 2,6-diaminopurine (DAP); Reversal by Adenine Sulfate.*

Addenda					
DAP (mg/l)	Adenine sulfate (mg/l)	No. of segments	Fresh wt of callus/segment (mg)	% of segments with buds	No. of buds/segment
—	—	30	79	97	3.9 ± .3†
—	80	27	82	100	6.6 ± .6
1	—	27	10	0	0 ± .0
1	80	30	85	100	5.1 ± .3
5	—	30	4	0	0 ± .0
5	80	30	22	47	.8 ± .2

* Readings made 39 days after start of test.
† Stand. error.

TABLE II. Inhibition of Budding and Callus Formation in Tobacco Stem Segments by 2,6-diaminopurine (DAP) (0.5 mg/l); Attempted Reversal with Various Compounds (3×10^{-4}M).*

Addenda		No. of segments	Fresh wt of callus/segment (mg)	% of segments with buds	No. of buds/segment
DAP	Compound				
—	——	21	108	100	4.5 ± .3†
—	Adenine	24	114	100	6.8 ± .7
—	Guanine	18	118	100	5.7 ± .5
+	——	24	57	46	1.4 ± .4
+	Adenine	24	120	100	6.3 ± .5
+	Arginine	21	65	57	1.4 ± .3
+	Guanine	24	76	67	2.1 ± .4
+	Hypoxanthine	21	82	86	2.5 ± .3
+	Thymine	24	55	58	1.2 ± .3
+	Xanthine	18	68	72	1.5 ± .3

* Readings made 37 days after start of test.
† Stand. error.

test media. The basal medium was modified White's solution as previously described(3) except that the level of KH_2PO_4 was increased from 12.5 to 37.5 mg/l and was made up with 1% Bacto-agar. The pH of each solution was adjusted to about 4.3 before autoclaving. Three stem segments were cultured aseptically on 50 ml of the medium in a 125-ml Erlenmeyer flask. Ten flasks or a total of 30 segments were prepared for each treatment.

On the control medium, these stem segments develop a white callus located mainly at the basal ends. This callus is easily cut from the old stem parts. In the experiment represented in Table I, the mean fresh weight of this callus after 39 days was 79 mg per segment. In the presence of 1 mg/l 2,6-diaminopurine, the fresh weight of the callus was reduced to 10 mg per segment. The fresh weight was only 4 mg in the presence of 5 mg/l DAP. A reduction in the thickness of the callus and the area covered by the callus occurred at both concentrations. In other experiments, callus formation was completely prevented by 50 mg/l DAP. Therefore it is obvious that diaminopurine does inhibit cell division in these cultures. On media to which 80 mg/l adenine sulfate had been added, the fresh weight of the callus was 82 mg per segment. This weight is not significantly greater than the control weight. In the presence of the adenine, 1 mg/l DAP did not decrease the fresh weight of the callus. However, 5 mg/l DAP gave a definite inhibition although it was less than that which occurred in the absence of adenine. Adenine, therefore, certainly alleviates the DAP inhibition of cell division in the tobacco stem callus.

Reversal of the DAP inhibition was attempted with several other compounds (Table II). Although hypoxanthine and guanine had a slight effect, these two compounds were not nearly as effective as adenine. Arginine and xanthine had little effect, if any, and thymine had no effect. The reversing action of adenine seems to be rather specific. On the

control medium, vegetative buds developed from the callus and internal phloem of the tobacco stem pieces(3,4). In the experiment cited in Table I, an average of 3.9 buds per segment were visible at the 39-day reading. The number of buds was reduced to zero when either 1 or 5 mg/l DAP was added to the basal medium. In another experiment (Table II), the number was decreased from 4.5 on the control medium to 1.4 on media containing 0.5 mg/l DAP.

In contrast to the callus growth which seemed little affected, bud formation was promoted by the presence of adenine in the basal medium(3). The number of buds was increased to an average of 6.6 per segment on media containing 80 mg/l adenine sulfate. DAP at a concentration of 1 mg/l reduced this value to 5.1 and at 5 mg/l to 0.8 bud per segment. Adenine thus alleviates the DAP inhibition of bud formation.

Autoclaving did not alter the effectiveness of the DAP in inhibiting bud formation and callus growth, nor did it affect the reversal by adenine since identical results were obtained when the DAP was Seitz-filtered and added to cooled autoclaved media as when it was added to the media before autoclaving.

Of the other compounds tested for reversing action, only guanine and hypoxanthine showed any activity (Table II). As with callus growth, these two compounds were much less effective than adenine in reversing the DAP inhibition of budding.

Cell division in habituated tobacco callus. The experiments with 2,6-diaminopurine have been extended to physiologically different types of cells. Strain No. 3137 of tobacco callus has been cultured continuously in our laboratories for 4 years and grows well on the same basal medium as is used for the culture of tobacco stem segments(3). Derived from tissues in which an exogenous source of auxin (indole-3-acetic acid or related compounds) was a requisite for growth, this strain may be called habituated since added auxin is not now necessary for its growth. That 2,6-diaminopurine markedly affects cell division in this type of callus is indicated by the following relative increases of fresh weight during a growth period of 28 days: control, 100; 0.1 mg/l DAP, 73; 0.5 mg/l DAP, 49; 1 mg/l DAP, 36; and 5 mg/l DAP, 17. Thus, diaminopurine inhibits cell division in both habituated and non-habituated tissues.

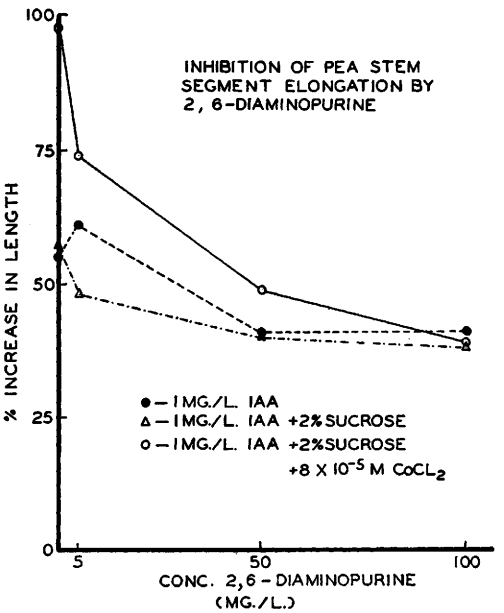

FIG. 1. Inhibition of pea stem segment elongation by 2,6-diaminopurine when added to various solutions of 1 mg/l indole-3-acetic acid (IAA), 2% sucrose, and 8×10^{-5}M $CoCl_2$. Readings made 24 hr after start of test. Original length was 5.3 mm.

Cell enlargement in tobacco stem pith pieces. Pieces of tobacco stem pith when cultured on the basal medium exhibit no cell division but enlarge greatly when supplied with indole-3-acetic acid (IAA)(5,6). In the experiment represented in Table IIIA, the cell enlargement as measured by increases of fresh weight was three times as great on a medium containing 2 mg/l IAA as it was on the control medium. This cell enlargement on media containing IAA was largely prevented by inclusion of 2,6-diaminopurine. DAP at 5 mg/l eliminated most of the enlargement. At concentrations necessary for reversal of the DAP inhibition, adenine itself was somewhat inhibitory. Nevertheless, reversal did occur. Data supporting this conclusion are presented in Table IIIB. The inhibition due to 5 mg/l DAP was prevented by 5 or 10 mg/l adenine. Also guanine • HCl at a level of 20 mg/l apparently reduced the effect of the DAP.

Cell enlargement in etiolated pea stem segments. Elongation of segments from etiolated pea plants may be used as a measure of cell

TABLE III. Inhibition by 2,6-diaminopurine (DAP) of Cell Enlargement in Tobacco Stem Pith Pieces; Reversal with Adenine.*

Addenda						
IAA (mg/l)	DAP (mg/l)	Adenine (mg/l)	No. pieces	Fresh wt/ piece (mg)	Increase fresh wt/ piece (mg)	Relative fresh wt increase
A.						
—	—	—	15	196	65	100
2	—	—	15	327	196	302
2	0.1	—	15	294	163	251
2	1	—	15	311	180	277
2	5	—	15	213	82	126
2	50	—	15	211	80	123
B.						
—	—	—	15	153	42	100
2	—	—	15	257	146	348
2	5	—	15	157	46	109
2	—	5	15	223	112	267
2	—	10	15	234	117	279
2	5	5	15	229	118	281
2	5	10	15	235	124	295
2	5+ (20 mg/l guanine · HCl)		15	193	82	195

* Weighings made 7 days after start of experiment. IAA = indole-3-acetic acid.

enlargement. Alaska No. 323 pea plants were grown in an *"Avena"* darkroom at a temperature of 25°C and relative humidity of about 88 per cent for 6-7 days. At this time sections 5.3 mm long were cut from the third internode just below the apex. Such sections, when floated for 24 hours on a solution of 2% sucrose and 1 mg/l IAA (pH 6.0) increased in length 57% (Fig. 1). Diaminopurine at 50 mg/l reduced the amount of elongation to about 40%. Growth in length was increased from the control value of 57% to a value of 98% of the original length when $CoCl_2$ (8×10^{-5}M) was added to the sucrose-IAA medium(7). DAP completely overcame this effect of cobalt and reduced growth to the same level as in the control segments. As may be seen from the dashed curve of Fig. 1, DAP reduced elongation when sucrose was not included in the test solution.

The rise in this curve at 5 mg/l DAP was not observed in subsequent experiments. The reductions in elongation in all treatments were correlated with lower fresh weights. Since the increase in length of the segments is the result of cell elongation, this process apparently is affected by DAP in pea stem segments. The inhibition due to DAP was prevented in part by addition of adenine, adenosine, or adenylic acid to the test solution (Table IV). At concentrations at which adenine prevented the inhibition, it itself was somewhat inhibitory to cell elongation.

Cell enlargement in Avena coleoptile segments. Segments, 5.3 mm in length, were cut from dark-grown (in the *"Avena"* room) oat coleoptiles 72 hours after soaking the seeds. These segments increased 87% in length when floated for 24 hours on a solution containing 2% sucrose and 2 mg/l IAA. The elongation was reduced to 52% when 100 mg/l 2,6-diaminopurine was included in the test medium.

Discussion. On the basis of the results obtained it may be concluded that cell division and cell enlargement in plants, and bud formation (involving both cell division and enlargement) can be inhibited by 2,6-diaminopurine. Furthermore, the inhibition may be almost completely if not entirely prevented by adenine. The results are in general agreement with those obtained with viruses, microorganisms, and animals by other investigators.

What the mechanism of DAP action may be is not known although the present results show that the DAP affects processes essential for both enlargement and division of plant cells. If the inhibitory action is via an interference with nucleic acid metabolism(8), then the participation of nucleic acids in cell enlargement is likely. From results obtained with *Lactobacillus casei,* Balis *et al.*(9) concluded that DAP inhibition is not through pentose nucleic acid but may be through

TABLE IV. Inhibition of Pea Stem Segment Elongation by 2,6-diaminopurine (DAP); Reversal by Adenine, Adenosine, and Adenylic Acid.*

Addenda					
DAP (mg/l)	Adenine (mg/l)	Adenosine (mg/l)	Adenylic acid (mg/l)	% elongation	Relative elongation
—	—	—	—	58	100
50	—	—	—	36	62
—	50	—	—	55	95
—	—	100	—	61	105
—	—	—	100	61	105
50	50	—	—	48	83
50	—	100	—	47	81
50	—	—	100	51	88

* 1 mg/l indole-3-acetic acid and 2% sucrose included in all solutions. Readings made 24 hr after start of test.

desoxypentose nucleic acid, coenzymes, or nucleotides such as adenosinetriphosphate. An effect on the last two types of compounds could interfere with the utilization of energy in plant growth(10,11).

Summary. 1. 2,6-diaminopurine has been shown to inhibit cell division in tobacco stem callus and habituated tobacco callus cultures, to prevent the formation of buds in tobacco stem pieces cultured *in vitro,* to reduce cell enlargement in tobacco pith pieces cultured *in vitro,* and to inhibit elongation of etiolated pea stem and *Avena* coleoptile segments floated on test solutions. 2. Adenine, arginine, guanine, hypoxanthine, and xanthine were tested in attempts to reverse the inhibition by diaminopurine of callus growth and bud formation in tobacco stem segments. Adenine was very effective and slight reversal was obtained with guanine and hypoxanthine. 3. Reversal of the DAP inhibition of tobacco pith cell enlargement was obtained with adenine and guanine, but adenine was more effective. No other compounds were tested on this tissue. 4. Reversal of the diaminopurine inhibition of etiolated pea stem segment elongation was attempted only with adenine, adenosine, and adenylic acid. All three compounds gave partial reversal.

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Received April 9, 1953. P.S.E.B.M., 1953, v83.