5. Sodium salicylate and sodium thiopentone depressed the ^{32}P uptake of 2:3-diphosphoglycerate and adenosine triphosphate to a greater extent than they depressed the ^{32}P exchange of inorganic phosphate and hexose monophosphate. These agents do not depress ^{32}P uptake by inhibiting triose phosphate dehydrogenase, for neither drug had any effect on glycolysis. The effects of 2:4-dinitrophenol and phenylbutazone resembled those of sodium salicylate and sodium thiopentone on the ^{32}P uptake of the phosphate fractions.

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Experiments on the Origin of Oak-Bark Tannin

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In earlier work (Hathway, 1958), it was concluded that the pyrogallol phenols are formed in oak leaves, translocated to the cambium and undergo oxidation there, and the resulting phlobatannin (which is a tail-to-tail polymer) is stored in the outer bark. In the present work an attempt has been made to study more closely the origin of 'tannins' in *Quercus pedunculata* Ehrh. Field experiments, which have been carried out throughout two seasons, were planned to throw light on (1) variation in the tannin content of stembark with age, (2) disturbances in the normal distribution of tannin in stembark, brought about by the ringing of the bark, (3) the translocation of phenolic precursors of the tannin in the sieve tubes of the phloem.

MATERIALS AND METHODS

Determination of the tannin in stembark. Young plants were obtained as required from Alice Holt nurseries, and the ages are calculated approximately from the time of the first transplantation. The entire stembark of each plant was deteched, dried to a 10% moisture content, and ground in a Wiley mill to 20-mesh. A portion of the bark was used for the analysis of moisture, and of tannin and non-tannin extractives by Grassmann, Endisch & Kuntara's (1951) semi-micro hide-powder method. Three different plants were utilized at 3-monthly intervals.

With older trees in the Forest of Dean, slivers of bark were detached from all sides of the main stem within a ring stretching from 3 to 4 ft. from ground level. The slivers were mixed and a representative sample was selected for analysis. Three different trees on each site were peeled in this way at 3-monthly intervals.

Method of ringing trees. During the first week in June 1957, two trees were selected on each site in Alice Holt forest, Hants, for bark-ringing experiments, and two further trees on each site for use as controls. A 6 in. ring of bark was detached at 1 ft. from ground level; care was taken not to impair the sapwood. All eight trees were felled during the last week in May 1958, when a sample of bark was immediately detached from the butt and crown of the main stem of each tree. Samples of stembark from the butts of the trees were detached from a cylinder, 1 ft. in height, immediately above the girdles on the ringed trees and from the corresponding location on the main stems of the controls. Samples of stembark from the crowns of the

trees were detached from a cylinder, 1 ft. in height, stretching from 6 in. below to 6 in. above a 3 in. exterior diameter of the main stem. The sixteen samples of bark were labelled in the field and slowly kiln-dried at 40° to a moisture content of 10–12%, at the Seasoning Department of the Forest Products Research Station, Princes Risborough, near Aylesbury, Bucks.

In the trees in the two foregoing experiments, a portion of the prepared stembark was ground in a Wiley mill to 20-mesh, and was used for the analysis of moisture, and of tannin and non-tannin extractives (*Official Methods of Analysis*, 1957).

Collection of sieve-tube exudate. Sieve-tube exudate was collected by cutting into the inner bark of the main stem of 28-year-old trees with a sharp knife. The trees selected were growing in a plantation in Alice Holt forest, and tapping cuts were made during July 1958. The outer bark was removed, and when the tapping cut penetrated the region of the functioning sieve tubes, which are located at a fraction of 1 mm. outside the cambium, there was an immediate exudation. If the knife was again passed along this cut, with the application of only slight pressure, the xylem was severed, and since this tissue was under subatmospheric pressure the exudate was immediately withdrawn with a hissing sound. When a second tapping cut is applied above the first one, it always yields exudate, whereas a second cut below the first one fails to effect exudation. Samples of exudate were transferred direct to previously prepared two-dimensional paper chromatograms, or they were collected in a small, stoppered test tube and immediately frozen in a freezing mixture contained in a Thermos flask.

Paper chromatography of translocation phenolics. Paper chromatography was carried out in all-glass apparatus in a constant-temperature enclosure at 25°. Chromatograms were dried at room temperature.

Sieve-tube exudate was spotted ($5 \times 5\,\mu$l.) at a distance of 2 cm. from both edges of the lower left-hand corner of Whatman no. 2 filter papers, 25·5 cm. square, and chromatographed by the ascending method with 6% (v/v) acetic acid containing 2% (v/v) formic acid as a first-way solvent system, and butan-2-ol–acetic acid–water (14:1:5, by vol.) as the second-way solvent system. Phenolics were detected by the $FeCl_3$–$K_3Fe(CN)_6$ and vanillin reagents (Hathway, 1958).

Isolation of catechins. Frozen sieve-tube exudate was thawed and applied to starting lines, 2 cm. from the lower edges of Whatman filter papers no. 3MM, 25·5 cm. square, and chromatographed by the ascending method with butan-2-ol–acetic acid–water (14:1:5, by vol.) for 20 hr. Longitudinal strips were cut from each paper and sprayed in order to locate (+)-catechin and (+)-gallocatechin on the unsprayed papers, which were then cut into transverse strips for elution with boiling ether in micro-Soxhlet apparatus. The respective solutions of the considerably purified substances were applied to single sheets of Whatman filter paper no. 3MM, which were chromatographed, excized and eluted as previously described. (+)-Catechin crystallized from hot water, forming needles, m.p. and mixed m.p. with an authentic specimen 173–175°. (+)-Gallocatechin crystallized from hot water, forming needles, m.p. and mixed m.p. with an authentic specimen (Hathway, 1958) 189–191°.

An aqueous extract of the phenolics of oak leaves. Mature sun leaves, harvested in July, were homogenized in ice-cold 80% methanol. The aqueous solution obtained after removal of chlorophyll and related compounds (Hathway, 1958) was used for chromatography.

RESULTS

Experiments were first carried out to determine whether the tannin content of the stembark varied during the season otherwise than as a result of increased age. For this purpose 6- and 8-year-old plants were investigated periodically from October until the following August. Each series of examinations involved different plants, and mean values are summarized in Table 1. In young plants, tannin accumulates during the period of active photosynthesis, and the enhanced level is maintained until the following spring. Thus 8-year-old plants contain more tannin in the stembark than 6-year-old plants.

In a second experiment, a number of older trees (from 50 to 100 years old) were investigated periodically from May until the following February. Each series of estimations was carried out on the butt regions of the stembark of different trees which

Table 1. *Mean values showing the seasonal variation of tannin in stembark*

Date	Site	Tannin (%) on moisture-free bark basis: 6-year-old plants	8-year-old plants
Oct.–Dec. 1956	Alice Holt nurseries	3·1	3·8
Jan.–Mar. 1957		3·1	3·8
Apr.–May 1957		3·2	3·9
June–Aug. 1957		3·8	4·5

Table 2. *Seasonal variation of tannin in the stembark of older trees*

Site	Age (years)	Height (m.)	Tannin (%) on moisture-free bark basis: May 1956	Aug. 1956	Nov. 1956	Feb. 1957
Forest of Dean	50	13·7	11·3	12·5	10·1	8·0
Blakeneyhill Woods	50	15·3	16·1	16·1	14·8	13·0
	50	15·3	11·9	12·3	13·1	10·0
Forest of Dean	60	15·3	8·9	9·2	7·6	10·0
Russell's Inclosure	60	15·3	6·9	7·2	5·4	8·0
	90	15·9	8·9	8·9	8·9	8·7

grew on two sites in the Forest of Dean. The values of the tannin analyses of all 24 trees are summarized in Table 2. Statistical analysis of these results shows that in trees of this age the observed seasonal variation is probably not significant. The marked differences between the tannin contents of trees which grew in the different localities are, however, significant. It is probable that, in the older trees, an increase in the tannin content of the bark tissue with increase in age is balanced by an increase in the (dead and living) bark tissue, with a result that the level remains without any significant variation throughout the season.

The effect of ringing on the distribution of tannin in stembark has been investigated on two sites in Alice Holt forest. Field experiments were commenced during the first week in June 1957, and terminated during the last week in May 1958. In this experiment, a 6 in. ring of bark was removed from the main stem of some 34-year-old trees, without damaging the sapwood. The leaves of the ringed oak trees turned colour very rapidly during autumn and, at the time of felling, shoots below the ring showed good bud formation and green leaves, whereas those shoots above the ring showed poorer bud formation, and carried either relatively few leaves which were ill-developed and red in colour or they were leafless. The red leaves contrasted with the luxuriant foliage of the controls. Tannin analyses of stembark derived from the butt and crown locations on the main stem are summarized in Table 3. Where the surviving trees showed leaf formation above the ring, analysis showed a high figure for extractable tannin above the girdle when compared with figures for the corresponding tissue of unringed trees of this age. Tannin analyses exceeding 17% have not previously been recorded for oak bark. For these trees, the difference in tannin content between the butt and crown is significantly greater than the corresponding difference in the controls. The fourth tree on each site had died before the end of the experiment, and the resulting low tannin contents agree in our experience with those obtained for bark which has undergone deterioration on the felled pole.

In order to determine whether tannin or its phenolic precursors is translocated by the sieve-tube cells of the phloem, the sieve-tube system of growing trees was tapped by cutting into the bark and the exudate obtained was applied direct to two-dimensional chromatograms, which showed the presence of six phenolics which gave the vanillin reaction. Compounds 1, 2 and 3 were found in the ethyl acetate-soluble fraction from oak bark by Hathway (1958); compound 2 was leucodelphinidin. Compounds 4 and 5, which were found in the ether-soluble fraction from oak bark by Hathway (1958), are (+)-gallocatechin and (+)-catechin respectively. The nature of compound 6 is unknown. Sieve-tube exudate therefore contains several phenolics of the bark, including the phenolic precursors of oak-bark tannin. The corresponding chromatogram of the methanolic extract derived from leaves (Hathway, 1958) shows that not all the phenolics which originate there are translocated in the sieve tubes, and in this respect the absence of ellagic acid and flavonoid compounds from the chromatogram of sieve-tube exudate is noteworthy. Ellagic and gallic acid do not occur in oak bark.

Table 3. *Effect of ringing on the distribution of tannin in stembark*

Forest	Site	Tree	Age (years)	Location on stem	Tannin (%) on moisture-free bark basis
Alice Holt	1	Control	34	Crown	12·7
				Butt	14·2
		Control	34	Crown	14·9
				Butt	16·3
		Bark ringed in June 1957 (this tree survived)	34	Crown	14·7
				Butt	18·1
		Bark ringed in June 1957 (this tree died)	34	Crown	10·6
				Butt	11·6
	2	Control	34	Crown	11·0
				Butt	12·5
		Control	34	Crown	14·3
				Butt	15·8
		Bark ringed in June 1957 (this tree survived)	34	Crown	14·0
				Butt	17·2
		Bark ringed in June 1957 (this tree died)	34	Crown	11·9
				Butt	12·5

DISCUSSION

Young plants do not show any significant variation in the tannin content of stembark, except for the progressive increase with increase in age, whereas in the older trees increase in tannin content of the bark with increase in age is balanced by an increase in the dead and living elements of the bark tissue. This suggests that the tannin undergoes further chemical changes in the dead elements of the outer bark, and this suggestion is confirmed by the work of Grassmann & Kuntara (1941), who found that the stembark of trees which were 100–150 and 200 years old contained less extractable tannin than that of 50-year-old trees, but that the level of tannin, soluble in aqueous sodium hydrogen sulphite, did not diminish with increase in age. This conclusion appears more reasonable than an assumption that the tannins participate in the metabolism of the tree, as would be implied by a statistically significant seasonal variation. Where observations have been confined to only one or two trees, however, a seasonal variation has previously been suggested for bark from *Quercus laevis* Walt. (Rogers, Calderwood & Beebe, 1950), *Picea sitchensis* Carrière and *Tsuga heterophylla* Sarg. (Clark & Andrews, 1921).

The results of the present study are consistent with the progressive increase in alkaloid content with increase in age of such alkaloid-containing plants as *Berberis darwinii* (Cromwell, 1933).

In the late seventeenth century, Malpighi's ringing experiments demonstrated that bark need not be present for the upward conduction of water, but must be present for the downward translocation of food (Thomas, Ranson & Richardson, 1956). As far as we are aware, the effect of ringing on the distribution of tannin in stembark has not, however, been previously studied. The work of Mason & Maskell (1928, 1929, 1934) established that continuity in the sieve-tube system in the bark is essential for the downward translocation of carbohydrates and nitrogenous substances. The trees on which our ringing experiments were made quickly lost their capacity for growth, and the leaves on the branches above the ring showed ageing. It was found that in the surviving trees the stembark tannin accumulated above the girdle. The possible translocation of tannin or its phenolic precursors in a downward direction (Hathway, 1958) therefore appears to be checked by the removal of the ring of bark. Unfortunately, these experiments do not elucidate the problem of the possible translocation of tannin or its precursors, as the high value for the tannin in the stembark above the ring may be due to obstruction of the downward translocation of tannin or to an increase in the formation of tannin resulting from an accumulation of the phenolic precursors in close proximity to the oxidase of the cambium.

For this reason, tapping cuts were made into the sieve-tube system of living trees by the method of Hartig (1860), and exudate was collected. There is plenty of evidence that the exudate obtained in this way is translocated material. Thus Hartig found that a second cut always yields exudate when it is applied above the initial one, but a second cut made below the first one fails to effect exudation. Münch (1930) found that sieve-tube turgor is released for a distance of several metres above a tapping cut. Finally, Ziegler (1956), who introduced hexoses into the bast of *Robinia pseudacacia* L., found them in the exudate below but not above the point of application. It should, however, be stated that slight contamination of the exudate from adjacent tissue cannot be excluded, though no exudation occurs until the actual sieve-tube system is tapped, and as the exudate is immediately transferred, the degree of contamination is considered to be extremely small. Zimmermann (1957, 1958) has recently used this method for the analysis of carbohydrates translocated in the sieve tubes of dicotyledonous trees. In the present work, exudate obtained in this way was found to contain several of the phenolics which had previously been found in the bark, including the phenolic precursors of oak-bark tannin. All the phenolics which originate in the leaves do not appear to be translocated in the sieve-tube system; ellagic acid and the flavonoids are conspicuous exceptions. In this connexion, the recently developed method (Kennedy & Mittler, 1953; Mittler, 1953, 1958) of using cut-off aphid stylets for obtaining sieve-tube translocation material is not applicable to older trees in which the sieve-tube system is protected by a thick layer of outer bark.

The evidence suggests that the pyrogallol phenols [(+)-gallocatechin and leucodelphinidin], which are formed in oak leaves, are translocated by the sieve-tube system to the cambium and undergo oxidation there, and the resulting phlobatannin, which was previously shown to be a tail-to-tail polymer (Hathway, 1958), is stored in the bark. The high level of tannin in ringed stembark therefore results from an accumulation of the phenolic precursors which undergo aerobic oxidation in the presence of cambial oxidase.

SUMMARY

1. In *Quercus pedunculata*, the tannin appears to be a waste product of metabolism, for it is localized in stembark tissue which does not participate in metabolic activities, and which is made up principally of dead elements.

2. In young plants, tannin accumulates during

the period of active photosynthesis and growth, and the enhanced level is maintained until the following spring. In the older trees, this progressive increase in tannin content with increase in age is balanced by increase in the dead and living bark elements, with the result that the level remains without apparent variation throughout the season. This suggests that the tannin undergoes further chemical changes in the dead elements of the outer bark.

3. The effect of ringing experiments on the normal distribution of tannin in the stembark of mature trees shows an accumulation of tannin above the girdle, suggesting that synthesis has taken place from phenolic precursors which are normally translocated downwards from the leaves.

4. Examination of the exudate obtained by tapping the sieve-tube system of growing trees shows the presence there of many of the phenolics found in the bark, including the pyrogallol precursors [(+)-gallocatechin and leucodelphinidin] of oak-bark tannin.

5. The evidence suggests that these pyrogallol phenols, which originate in the leaves, are translocated by the sieve-tube system to the cambium and undergo oxidation there, and the resulting phlobatannin is stored in the bark.

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Substrate Specificity of Phosphoprotein Phosphatase from Spleen

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Recent investigations from several Laboratories have revealed the presence, in mammalian tissues, of an enzyme system for the dephosphorylation of phosphoproteins, which appears to be distinct from phosphomonoesterases (Feinstein & Volk, 1949; Norberg, 1950; Mattenheimer, 1953; Thoai, Roche & Pin, 1954; Sundararajan & Sarma, 1954, 1957). Evidence for the specific nature of the enzyme has largely been based on its inability to split β-glycerophosphate, a typical phosphomonoester substrate. A detailed study of the substrate specificity of this enzyme is warranted in view of the recent observation of Perlmann (1955) that phosphoproteins containing phosphomonoester linkages are susceptible to dephosphorylation by phosphomonesterases. The present paper describes the results of an investigation on the specificity of highly purified preparations of phosphoprotein phosphatase from ox spleen towards a number of substrates representing different types of phosphorus linkages. As a parallel study, the specificity of a partially purified preparation of the enzyme