identified TxB_2 in the bathing medium. After extraction and isolation of TxB_2 by high-pressure liquid chromatography, we obtained by combined gas chromatography and mass spectrometry the expected mass spectrum of the methyl ester of the methoxime trimethylsilyl ether derivative (*6*). The quantity of TxB_2 formed in the bath was determined by using octadeutero-TxB_2 as a carrier and selectively monitoring the ratios of *m/e* of the ions 301 and 304 (*7*). Three minutes after PGH_2 (255 n*M*) was added to the muscle bath containing 2.95 ml of buffer and 0.05 ml of the platelet particulate suspension, the muscle bath was found to contain 52 n*M* TxB_2.

Having shown that the TxA_2 generating system produced a contraction similar to that evoked by aggregating platelets, we assessed the formation of TxA_2 by whole platelets aggregated under these conditions by measuring TxB_2 in the aggregation module at various times after the addition of thrombin. If one assumes that the reaction follows first order kinetics, the rate of TxB_2 formation should parallel the concentration of TxA_2. The rate of TxB_2 formation reached a maximum in less than 15 seconds after thrombin addition to the platelets and essentially ceased after 3 to 4 minutes (Table 1). Thus the time course of the contractions produced by aggregating platelets are a reflection of the concentration of TxA_2, indicating concomitant variation in the levels of the putative mediator and the response.

We conclude that TxA_2 released from aggregating platelets can contract coronary smooth muscle in vitro. These findings provide the basis for the following hypothesis: Platelet aggregation in areas of damaged endothelium can release TxA_2 and thus cause constriction of large coronary arteries. This hypothesis merits testing in conditions where coronary spasm is known or postulated (initiation of myocardial infarction, sudden cardiac death, and variant angina).

More generally, these findings suggest that TxA_2 may be a factor in other forms of arterial constriction in which platelets participate. The cerebral arterial spasm associated with subarachnoid hemorrhage is of particular interest, as we have found that the TxA_2-generating system produces contraction of bovine intracranial arterial strips (*8*).

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8 April 1976; revised 6 July 1976

Microbial Degradation of Condensed Tannins

Abstract. *A strain of* Penicillium adametzi *Zaleski was isolated from enrichment cultures with condensed tannins as the carbon source. Low-molecular-weight condensed tannins, extracted and purified from* Pinus radiata *bark, were used as substrates for quantitative growth measurements on this fungus in defined culture conditions.*

The well-known ability of plant tannins to inhibit the growth of microorganisms has been attributed to the capacity of these substances to bind strongly to proteins and polysaccharides. Enzymes are wholly or partially inactivated by complex formation with tannins (*1*), while potential microbial substrates such as polysaccharides and nonenzyme proteins become highly resistant to microbial attack after binding to tannin molecules (*2, 3*). The significance of these effects of tannins in relation to plant disease resistance and to the rates of decomposition of organic molecules in soil was reviewed by Starkey and his colleagues (*2, 3*), who also found that several of the condensed tannins were themselves very resistant to microbial attack. It is nevertheless evident that microorganisms capable of degrading such a ubiquitous natural product must exist, although, considering the obvious importance of such organisms in soil biochemistry and microbial ecology, it is perhaps surprising that very little work has been published in this area (*4*). I describe here for the first time the isolation of an organism capable of growth on condensed tannins as sole carbon source under defined culture conditions. This work forms part of a study on the microbial breakdown of *Pinus radiata* bark, a major waste product of the New Zealand forestry industry. Condensed tannins and related phenolic polymers account for 50 to 60 percent of the dry weight of this bark (*5, 6*).

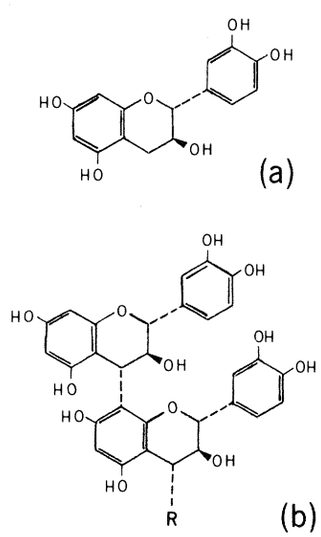

Fig. 1. Structures of (a) (+)-catechin and (b) the procyanidin dimer, B-3 (R = H), and the trimer, C-2 [R = (+)-catechin].

Enrichment culture experiments were conducted with a purified preparation of condensed tannins (*7*) as sole carbon source in a mineral salts medium (*8*). Temperature, *p*H, tannin concentration, nitrogen source, and time of incubation were varied in repeated attempts to isolate tannin-degrading organisms from several forest and garden soil samples, and from samples of rotting wood and bark. Ultimately an inoculum from a rotting *Pinus radiata* log yielded a filamentous fungus apparently capable of growth on condensed tannins as its sole carbon source. This fungus has since been classified by the Commonwealth Mycological Institute, Kew, London, as *Penicillium adametzi* Zaleski, and has been given the catalog number IMI 198150.

Although it is now generally accepted that condensed tannins are polymers of flavan-3-ols such as (+)-catechin (Fig. 1a) or flavan-3,4-diols such as leucocyanidin (*9*), no individual high-molecular-weight condensed tannin polymer has ever been isolated in the pure state. Previous microbiological studies with these substances (*2–4*) have used partially purified plant extracts, that is, tannin mix-

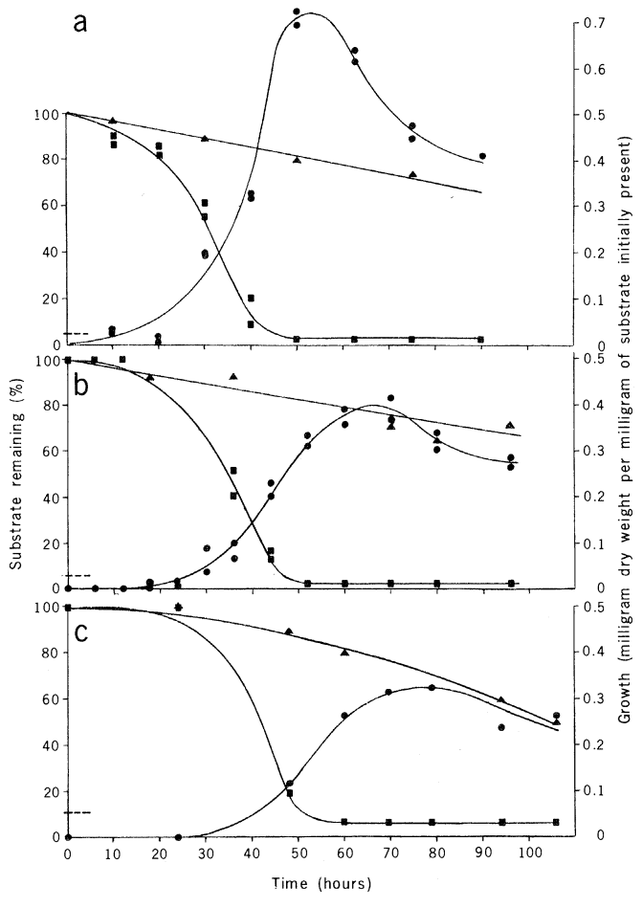

Fig. 2. Growth of *Penicillium adametzi* isolate on (a) (+)-catechin, (b) procyanidin B-3, and (c) procyanidin C-2. ●, Mycelial dry weight; ■, percentage of substrate remaining in culture filtrates; ▲, percentage of substrate remaining in uninoculated control flasks; and ---, limit of estimation of substrate. The polyphenol concerned was dissolved to 0.1 percent in a mineral salts solution (*8*). The medium was adjusted to *p*H 5.1, sterilized by filtration, and then dispensed into 5-ml lots in sterile 25-ml flasks. Each flask was inoculated with a single loopful of a fresh spore suspension of the *Penicillium* and incubated statically at 28°C with several uninoculated controls. Flasks were removed at intervals and the contents assayed for cell material and residual substrate (*15*).

tures of the type used in the above isolation experiments. This is unsatisfactory from a biochemical viewpoint since so little is known about the structures and properties of individual tannins in such mixtures. It has been reported, however, that condensed tannins are represented at the lower end of their molecular weight range by the oligomeric proanthocyanidins, the chemistry of which is now well understood (*10, 11*). In *Pinus radiata* bark several proanthocyanidins occur along with much larger quantities of the polymeric tannins (*5, 12*), and I therefore chose two of these compounds as "model" condensed tannin substrates for growth studies with the isolate of *P. adametzi*. Chemical structures of the trimeric procyanidin C-2, the dimeric procyanidin B-3, and the fundamental tannin monomer (+)-catechin are shown in Fig. 1; details of the extraction and purification of these substances have been summarized (*13*).

Growth experiments in which catechin and the procyanidins B-3 and C-2 were used as sole carbon substrates for the fungus were carried out (see Fig. 2). Flasks were incubated statically since vigorous aeration increased the rate of oxidation of the substrates to polymerized products. The results in Fig. 2 show conclusively that *P. adametzi* is capable of relatively rapid growth in a defined medium with the low-molecular-weight tannins procyanidin B-3 and C-2 as sole carbon sources. Lower growth yields than the maximum shown in Fig. 2c were obtained in other experiments with the trimer C-2, and it may be that the generally lower growth responses from the procyanidins in comparison with that from catechin reflect some degree of tannin-type binding of the procyanidin substrates to fungal polymers. Procyanidins, but not catechin, are capable of forming complexes with proteins (*14*).

Although procyanidins B-3 and C-2 are true tannins in that they will precipitate gelatin from solution (unpublished observations), an objection could be raised that they might not be strictly representative of condensed tannin polymers because of their low molecular weight. I therefore examined the growth of the *Penicillium* on higher molecular weight tannins prepared as described above (*13*), dissolved to 0.03 percent in the mineral salts medium (*8*) and sterilized by filtration. While growth of static cultures was poor, vigorously sparged cultures yielded 0.22 to 0.28 mg (dry weight) of cell material [obtained from mycelial nitrogen determinations (*15*)] per milligram of tannin originally present in the medium. Although these yields appear low compared with those obtained with the procyanidins, they are, in fact, an underestimate since, under the vigorous aeration conditions used, some of the tannin substrate was oxidized to insoluble polymerized products over the period of incubation of 7 to 10 days (*16*). Binding of tannins to mycelial filaments, clearly observable in these cultures, probably reduced still further the amount of substrate available for metabolism by the fungus.

The capacity of *P. adametzi* to degrade condensed tannins has thus been established. Although the biochemical pathway by which this is accomplished is unknown, it is likely that at least the first enzyme involved would possess some unique properties, since enzyme-tannin interactions are usually strong, apparently nonspecific, and result in at least partial inactivation of the enzyme concerned (*1*).

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13. Finely ground *Pinus radiata* bark, previously extracted with hexane and then with toluene, was exhaustively extracted with ethyl acetate. Much of the higher molecular weight tannin material in the extract was removed by precipitation, first by concentration on a rotary evaporator at 30°C, then by fractional precipitation with hexane [T. A. Geissman and H. F. K. Dittmar, *Phytochemistry* **4**, 359 (1965)]. Catechin, procyanidins, and remaining higher molecular weight tannins were extracted into water and then filtered on a column of Sephadex G-25 (Medium) (88 by 5 cm), prepared and eluted with 50 percent aqueous acetone [L. J. Porter and R. D. Wilson, *J. Chromatogr.* **71**, 570 (1972)]. The early fractions (excluded from the gel) contained only the higher tannins and were freeze-dried for use in later growth studies. This preparation was shown by paper chromatography to be carbohydrate-free. The procyanidins and catechin were subsequently separated from one another by chromatography on a column of Sephadex LH-20 (74 by 2.6 cm), prepared and eluted with methanol. Traces of other phenolics in the preparations were removed by partition chromatography on columns of cellulose which were partially eluted with a mixture of butan-2-ol, acetic acid, and water (14:1:5) (*11*), then extruded and sliced [A. Thompson, in *Methods in Carbohydrate Chemistry*, R. L. Whistler and M. L. Wolfrom, Eds. (Academic Press, New York, 1962), vol. 1, p. 36]. After a final cleanup of each preparation by gel filtration on Sephadex G-25, they were then tested for homogeneity by two-dimensional paper chromatography (*11*) and freeze-dried. Yields were 0.26 g (catechin), 1.51 g (B-3), and 0.17 g (C-2) from 1 kg of bark.
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15. Flask contents were filtered through Whatman paper No. 54. Filtrates were adjusted to 5.0 ml and frozen. Mycelia were washed five times with 5.0 ml of distilled water each time at 40°C, dried overnight at 110°C, and then analyzed for total cell nitrogen by a Kjeldahl procedure. Figures for mycelial dry weights were obtained from a calibration curve relating dry weight to total cell nitrogen prepared with the *Penicillium* grown on glucose in the salts medium, using the harvesting and washing procedure already described. Residual catechin or B-3 in culture filtrates and in uninoculated controls was assayed by gas-liquid chromatography of their trimethylsilyl (TMS) derivatives according to a method adapted from that of Eastmond [R. Eastmond, *J. Inst. Brew. (London)* **80**, 188 (1974)]. As the TMS derivative of the C-2 trimer was insufficiently volatile for such an assay, C-2 was estimated by semiquantitative paper chromatography of samples of the filtrates with a range of standards on the same paper. Two-dimensional paper chromatograms of all culture filtrates showed that interference in the assays by traces of other phenolic products was negligible.
16. Fifty to 60 percent of the tannins [M. J. Taras, A. E. Greenberg, R. D. Hoak, M. C. Rand, Eds., *Standard Methods for the Examination of Water and Waste Water* (American Public Health Association, Washington, D.C., 1971), p. 346] in uninoculated control flasks was precipitated from solution after 7 days of incubation and aeration.
17. I thank Cushla McMurtry for her technical assistance, and Dr. Lawrence J. Porter for his advice and for providing samples of procyanidins for reference purposes.

20 February 1976; revised 18 May 1976

Behavioral Fever in Newborn Rabbits

Abstract. *Rabbit pups, 12 to 72 hours old, did not develop a fever when injected intraperitoneally with a pyrogen and maintained at an ambient temperature of 32°C for 2 hours. When placed in a thermally graded alleyway, animals injected with pyrogen selected gradient positions that represented significantly higher temperatures than controls injected with saline (40.4° in contrast to 36.4°C). Allowing the pups to remain at their selected positions for 5 minutes caused a significant increase in the rectal temperatures of the pyrogen-injected pups but not that of the controls. Thus, newborn rabbits will develop a fever by behavioral means after a single injection of an exogenous pyrogen.*

Unlike adults, newborn mammals do not develop a fever upon initial exposure to a bacterial pyrogen (*1*). Neonates do not respond well to thermal stresses in general, and can maintain normal body temperatures only within a narrow range of environmental temperatures. In this respect, infant mammals are very much like reptiles, which have inadequate or nonexistent physiological thermoregulatory mechanisms. Reptiles, however, will maintain their body temperatures within narrow limits when given the opportunity to do so behaviorally (*2*). Many infant mammals also show thermal preferences from the day of birth and will maintain normal body temperatures by moving to the appropriate place in a thermal gradient (*3*). In response to a bacterial infection, iguanas will develop a fever by spending more time at higher environmental temperatures (*4*). Fish also show a behavioral fever when injected with a pyrogen (*5*). We report here that, in response to an initial challenge with a pyrogen, newborn rabbits, unable to develop a fever physiologically, will do so behaviorally by selecting higher environmental temperatures than nonchallenged controls do.

Eleven New Zealand white rabbit pups from two litters were used in these experiments. They were 12 to 72 hours old and weighed between 50 and 89 g. The pups were removed from their mothers and divided into three groups matched as closely as possible for body weight. One group was given an intraperitoneal injection of *Pseudomonas* polysaccharide (Piromen), 500 μg per kilogram of body weight, dissolved in sterile saline (250 μg/ml). The second group received the saline vehicle alone (2 ml/kg), and the third group was given no treatment. After injection, the pups were placed in individual containers in an incubator kept at 32°C. Two hours after injection, rectal temperatures were measured with a 36-gauge, copper-constantan thermocouple inserted 2.0 cm into the rectum. The pups were then placed, two at a time, in a temperature gradient apparatus similar to that used by Ogilvie and Stinson (*3*). This consisted of an alleyway whose bottom was a copper bar (183 by 15 by 0.64 cm) with aluminum sides (122 cm long, 15 cm high) and a hinged Plexiglas top. At each end of the alleyway, the bar extended 31 cm. Heating tape was wrapped around one end, and temperature was controlled with a Variac voltage transformer. The temperature gradient along the alleyway ranged from 22° to 55°C. Thermocouples were placed every 2.5 cm along the gradient, and 49 divisions were marked along the sides of the enclosure corresponding to the thermocouple placements. The pups were placed in the gradient with their noses touching a point corresponding to 30°C; half were positioned facing the hot end and half facing the cool end. The position of each animal in the gradient was recorded every minute, as were the temperatures under the thermocouples. An experiment, which generally took no more than 25 minutes, was terminated when a pup remained at the same place in the gradient for at least 5 minutes. When a pup was removed from the gradient, its rectal temperature was again recorded, and it was returned to its mother. No rabbit in any group was given more than a single injection of Piromen. The rabbits getting no injection on day 1 received Piromen on day 2 and saline on day 3; those receiving saline on day 1 got no injection on day 2 and Piromen on day 3.

To ensure that the dose of Piromen used was sufficient to give older rabbits a fever, the pups were divided into two groups, matched for body weight, when they were 14 days old. Five were injected with Piromen (500 μg/kg) and six with a similar volume of saline alone. All the pups were maintained individually in the incubator at 27°C, and rectal temperatures were taken before and 2 hours after injection.

All data are reported as the mean (± standard error of the mean) unless otherwise indicated. Student's *t*-tests were performed on the data, and the null hypothesis was rejected when $P \leq .05$.

The main results of the experiment are shown in Fig. 1a. Rabbit pups injected with saline selected a gradient temperature of 36.5° ± 0.47°C. Pups injected with Piromen selected a significantly higher gradient temperature,