was not affected, precluding the interpretation that the tumour exhausted an essential component of the medium. This possibility is further made unlikely because liver explants cultured in the presence of tumour tissue consistently increased in total mass and protein by 15–20%, whereas controls did not.

All culture dishes containing Walker carcinoma (and those serving as controls) were supplemented with additional 2 mM glutamine (in view of the high glutaminase content of tumours) and with hydrocortisone (0.4 μg ml^{-1}). The inhibitory effect of the tumour on the rate of enzyme accumulation in the liver explants did not depend on the presence of the corticoid in the medium; rather, in initial mixed culture experiments without hydrocortisone, enzyme levels in the cocultured liver explants were too low to be measured accurately.

Table 1 Enzyme development in 19-d foetal liver explants

Days in culture	Foetal age	None: Injected	None: Controls	Cortisol (0.4 μg ml^{-1}): Injected	Cortisol (0.4 μg ml^{-1}): Controls
		TAT			
0	19	0.03	0.03	0.03	0.03
1	20	—	—	—	—
2	21	0.31	0.14	0.74	0.28
3	22	0.54	0.23	1.06	0.67
4	23	0.44	0.39	1.25	0.95
		AAT			
0	19	23.7	18.3	23.7	18.3
1	20	—	—	—	—
2	21	48.2	30.0	64.3	42.0
3	22	66.5	41.8	78.1	62.7
4	23	60.5	56.6	92.6	76.0
		Arginase			
0	19	26	21	26	21
1	20	37	13	62	47
2	21	90	32	142	90
3	22	83	59	217	158
4	23	70	71	248	174

Additions to culture medium.

Foetuses in one uterine horn were injected with 25 μg cortisol each, 24 h before their livers were explanted as described in the legend to Fig. 1; non-injected foetuses from the other uterine horn served as controls. Activities are given in units per g tissue.

To ascertain whether the inhibition of TAT and AAT accumulation was tumour specific, foetal livers were cultured in conditions identical to those described, but with mammary tissue from a pregnant dam instead of tumour. The activities of all three enzymes were always 10–20% higher than in controls (foetal liver explants alone). We cannot explain this observation; however, a differentiating effect of mammary mesenchyme on tumour tissue explants has been reported[14].

The inhibitory effect of the tumour on the enzymatic maturation of the liver explants was reversible and depended on the continued survival of the tumour in culture. When the sponges supporting the tumour tissue were replaced by empty sponges on day 3 of culture, enzyme accumulation resumed at the rate of controls. Similarly in some experiments in which the tumour did not survive as long as the liver explants (as ascertained by thymidine kinase activity measurements), accumulation of the indicator enzymes resumed at the rate of controls.

Because the cultures containing the tumour had to be maintained in the presence of hydrocortisone to obtain detectable enzyme levels in the cocultured livers, and all three enzymes responded to the hormone with an increased rate of accumulation in organ culture although two of them did not respond in this way *in utero*, it seemed that the corticoid may serve some nonspecific function in the system, perhaps the maintenance of tissue viability, similar to that of certain serum factors[15]. To test this possibility we injected each of several foetuses *in utero* with 25 μg hydrocortisone 24 h before their livers were explanted into media with and without hydrocortisone. The results of a representative experiment (Table 1) show that enzyme levels in cultures from 19-d-old foetuses which had received hydrocortisone *in utero* were similar for 2–3 d in culture to those which were explanted from uninjected controls into medium containing hydrocortisone. The rate of enzyme accumulation in the injected samples then slowed, whereas explants maintained on hydrocortisone with daily medium changes continued to respond with the same rate of enzyme accumulation; those injected and maintained on hydrocortisone showed the greatest response, particularly arginase. When foetuses were injected with hydrocortisone on day 16 of gestation and their livers were explanted 24 h later into media with and without hydrocortisone, the injected hormone was ineffective (data not shown). This argues against the possibility that the injected hormone had some lasting, nonspecific effect; rather it suggests that hydrocortisone has a specific inducing effect if administered when the liver is competent to initiate *in utero* the processes which culminate in enzyme production *in vitro*.

Our findings suggest that the Walker 256 carcinoma produces a diffusable factor (or factors) with an inhibitory effect on the rate of accumulation of some, but not all, enzymes that develop in embryonic liver maintained in organ culture. The effect is specific for Walker carcinoma tissue and its maintenance depends on the continued survival of the tumour in culture. The apparent rapid inactivation of the factor(s) in this system need not signify an inherent instability precluding its isolation; serum factors that withstand remarkably harsh conditions during their purification are known to be inactivated rapidly by cells in culture[16]. While the endocrine state of a tumour-bearing animal is undoubtedly modified, our results show that Walker carcinoma tissue exerts a readily measurable direct effect on developing liver in organ culture, that is without the intervention of endocrine changes. The system described is therefore well suited as an assay for the isolation of the pertinent factor(s) and for the study of its mechanism of action.

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Mechanism for suppression of cellular biosynthesis of prostaglandins

BIOSYNTHESIS of prostaglandins (PGs) from unsaturated fatty acid precursors involves a complex sequence of reactions that seem to proceed rapidly in response to physiological stimuli. Studies *in vitro* have revealed a capacity for biosynthesis greater than would be expected from measures of tissue content[1,2] or daily prostaglandin (PG) production[3]. Biosynthesis requires

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release of esterified precursor from tissue lipids[4,5], and control of the hydrolytic event may be a major means of controlling PG biosynthesis (for example, in brain[6] or spleen[7]). Another possible type of regulation is control of cyclo-oxygenase activity. Many chemical agents have been examined as modifiers of the PG-forming oxygenase (for example, reviews in refs 8 and 9). The enzyme can be inhibited by fatty acids[10] although they appear only in limited amounts in the cytosol. In addition, inhibition of the oxygenation reaction has been observed *in vitro* with added glutathione peroxidase (GSP) and reduced glutathione (GSH)[11–14]. We have further investigated this form of enzymatic regulation, and propose that it inhibits by destroying an essential activator of the oxygenase which forms the PGs and thromboxanes.

Supernatant (BSN) obtained from centrifugation of homogenates of bovine vesicular glands at 105,000*g* for 90 min gave 60% inhibition of oxygenation of arachidonic acid by bovine vesicular gland microsomes, and 40% inhibition of the activity of an acetone powder preparation from sheep vesicular glands. Since the bovine oxygenase showed only 12% of the specific activity of that from sheep, and the supernatant from sheep vesicular glands gave little inhibition of oxygenase preparations, the most active inhibitory and oxygenating preparations were used in subsequent experiments (Table 1). When sodium cyanide, an inhibitor of PG synthesis[15,16], was in the oxygenase assay mixture at a concentration (0.5 mM) that gave about 45% inhibition of oxygenation in the absence of added BSN, a synergistic inhibition was observed that was proportional to the amount of BSN when < 0.1 ml of BSN was present, and was complete with ≥ 0.1 ml of BSN. The BSN was only about half as inhibitory when additional GSH was omitted from the assay, and did not inhibit the oxygenase when 1.0 mM *N*-ethylmaleimide (NEM, which combined with the necessary mercaptan group of GSH) was added. Oxygenase activity could also be recovered when NEM was added to a reaction mixture several minutes after total inhibition by 0.1 ml of BSN. The total extent of reaction before and after addition of NEM was the same for all concentrations of BSN used, indicating that the capacity of the oxygenase for production of PGs and related derivatives was not irreversibly impaired by BSN.

When radioactive eicosatetraenoic acid (150 nmol) was incubated with the oxygenase preparation, 69–74% of the product formed migrated with ^{3}H-PGF$_{2\alpha}$ (R_f = 0.05–0.20) and ^{3}H-PGE$_2$ (R_f = 0.20–0.45) standards, 12–17% with the region associated with PGD$_2$, PGG$_2$ and PGH$_2$ (R_f = 0.30–0.45) and 10–19% with the hydroxy acid standard (R_f = 0.45–0.60). The relative amounts of product formed were similar in the presence of GSH and the BSN with or without NEM present. In the presence of 4 mM S$_n$Cl$_2$, 73–80% of the product migrated with PGF$_{2\alpha}$ (R_f = 0.05–0.20) and 6–19% with PGE$_2$ (R_f = 0.20–0.29) standards.

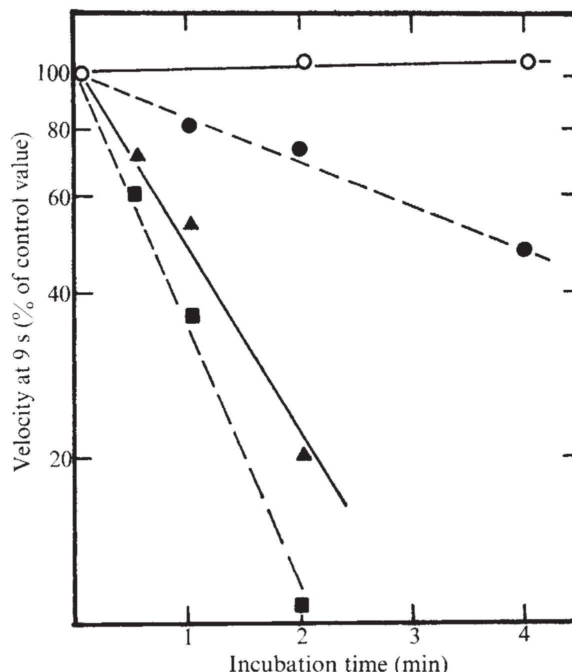

Fig. 1 Time-dependent destruction of the oxygenase activator by BSN and GSP. Activator was generated from 65 μM arachidonic acid with 350 μg of sheep vesicular gland dioxygenase in 3 ml of 0.1 M Tris chloride at *p*H 8.5, containing 0.67 mM phenol and NaCN, as indicated below. After 4 min of reaction and cessation of oxygen consumption, 500 μM GSH and the BSN or GSP solution were added. Then, after various times, the oxygenase reaction was initiated by addition of 350 μg of vesicular gland enzyme, and initial velocities were measured using an oxygen electrode equipped with an electronic differentiator. The value for the oxygenase velocity at 9 s is a measure of the amount of activator[16,18]. ○, 0.5 mM NaCN+heat-treated GSP; ●, 0.5 mM NaCN+120 U of GSP; ■, 1.0 mM NaCN+150 U of GSP; ▲, 0.5 mM NaCN+0.015 ml of BSN.

We have proposed[14,16] that the oxygenase required a hydroperoxide activator and PG production may be inhibited by removal of activator molecules by GSP. Figure 1 shows that very low levels of either BSN or GSP destroy the activator in a few minutes.

The inhibitory agent in the cytosol of bovine glands (BSN) had several properties similar to those of the GSP purified from rat liver[17] or bovine blood[18]. Both GSP and BSN were dependent on added GSH for maximal inhibitory ability (and added NEM,

Table 1 Inhibition of sheep vesicular gland dioxygenase by BSN

Initial reaction conditions	After addition of dioxygenase		After addition of 1 mM NEM	
	Optimal velocity (μmol O$_2$ min^{-1} mg^{-1})	Extent (μmol O$_2$)	Optimal velocity (μmol O$_2$ min^{-1} mg^{-1})	Extent (μmol O$_2$)
Control	470	46	0	0
+50 μl BSN	430	41	50	7
+100 μl BSN	270	28	145	16
+0.5 mM NaCN	289	45	0	0
+0.5 mM NaCN+50 μl BSN	182	29	74	16
+0.5 mM NaCN+70 μl BSN	89	19	161	22
+0.5 mM NaCN+100 μl BSN	0	0	239	38
+0.5 mM NaCN+100 μl heat-treated BSN	222	32	124	12
+0.5 mM NaCN+100 μl BSN+1 mM NEM	318	47	0	0
+0.5 mM NaCN+100 μl BSN+no exogenous GSH	205	43	0	0
+0.5 mM NaCN at *p*H 7.0	420	42	0	0
+0.5 mM NaCN+70 μl BSN at *p*H = 7.0	277	29	17	22

BSN was obtained by homogenising 100 g of bovine seminal vesicles in 200 ml of 0.1 M Tris-HCl at *p*H 8.5. The homogenate was centrifuged at 10,000*g* for 15 min and the supernatant was filtered through cheesecloth. This supernatant was centrifuged for 90 min at 105,000*g* and the resultant supernatant was removed and stored at −20 °C for later assay. Assays were made in 3.0 ml of 0.1 M Tris-HCl at *p*H 8.5 (except where otherwise indicated the *p*H 7.0 buffer was 0.1 M EDTA) containing 0.67 mM phenol, 0.5 mM reduced glutathione, 65 μM arachidonic acid and other additions as indicated. Reaction was initiated by addition of 375 μg of phenol-activated sheep vesicular gland enzyme. After 3–5 min, 1.0 mM *N*-ethylmalemide was added to the reaction mixtures. Dioxygenase activity was monitored with an oxygen electrode equipped with an electronic differentiator.

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which removes GSH, eliminated inhibition by both); inhibitory in a concentration-dependent manner; synergistic with NaCN in the inhibition of PG formation; heat-labile and non-dialysable, destroying the activator of the oxygenase in a time-dependent manner (Fig. 1).

BSN also appeared most effective as an inhibitor at or near the *p*H optimum for GSP (*p*H > 8.5; see also refs 19–22). There was a slight difference in the amount of $(NH_4)_2SO_4$ required to salt the two inhibitors out of solution: approximately 68% of the total inhibitory ability of the BSN precipitated from solution between 0.33 and 0.50 saturation with $(NH_4)_2SO_4$ whereas about 60% of the measured GSP activity of rat liver supernatant was salted out between 0.45 and 0.65 $(NH_4)_2SO_4$. (Rat liver–GSP was obtained by extraction of protein precipitated in ethanol at −25 °C (ref. 17), whereas the BSN was a high speed supernatant and contained more protein per ml than the liver preparation which could explain the differences.) BSN, like GSP, catalysed the destruction of cumene hydroperoxide in the presence of reduced GSH (8 U peroxidase activity per μl of BSN—see ref. 18 for assay conditions and definition of units).

The apparent K_m for GSH may vary greatly depending on the initial steady-state concentration of the peroxide substrate[22,23], suggesting that as the local physiological concentrations of hydroperoxide substrate approach 1μM the *in vivo* level of GSH (usually between 100 μM and 5 mM (refs 24 and 25) may be close to its K_m for GSP. Thus, metabolic alterations of the intracellular concentrations of GSH within this concentration range could alter directly the effective peroxidase activity and thereby act as an intracellular modulator of the activity of the fatty acid oxygenase(s) that form the PGs and thromboxanes. Homogenisation of a tissue *in vitro* also gives dilution of the cytosolic GSP and GSH to concentrations that are less inhibitory than those *in vivo*.

Formation of PGs *in vitro* has been reported to be inhibited in a dose-dependent manner by a component of the supernatant obtained during the preparation of microsomes from bovine seminal vesicles[26,27] and rabbit renal medulla[28], but the nature of the inhibition was unclear. Inhibition by a soluble component from tissue preparations may also be inferred from the data from other studies with these and other tissues[29–32], that is, total product found in one or more of the PG fractions was significantly less when supernatant was included in the assays. Metabolic enzymes could lower accumulated levels of PG, particularly where the assay involves measurement of final concentrations of PG products (for example, ref. 32).

Although there is insufficient evidence to propose that GSP is the only inhibitor present in supernatant preparations, many assays for PG biosynthesis have included levels of GSH > 0.2 mM (refs 28–31), and a GSP-like activity could account for much of the inhibitor in supernatant reported in some of those *in vitro* studies. The observed effectiveness of the GSH-dependent peroxidase in blocking PG biosynthesis suggests that it has a significant physiological role in the many cell types in which it occurs. Thus, peroxidase activities could suppress PG biosynthesis by destruction of the essential activator as it is formed, so that (depending on the levels of peroxidase and essential cofactor, GSH) some dioxygenase molecules are not activated and therefore cannot participate in PG production. A decreasing concentration of cellular GSH could be an important means for cells to modulate cyclo-oxygenase activity to give increased production of PGs and their related derivatives.

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Evidence for the proximity of sweet and bitter receptor sites

Although sweetness and bitterness are generally referred to as two of the common or basic tastes, it is by no means certain that they are, in fact, primary[1]. Furthermore, neurophysiological evidence has shown that response to several of the basic taste stimuli can occur in a single taste cell[2]. Much structural progress in the chemistry of sweetness has been made in the past decade through Shallenberger's hypothesis[3–5] that the sweetness of sugars depends on hydrogen bonds between the sugar molecule and receptor site. Our previous work[6–9] has indicated that sugar molecules and their simple derivatives are nearly always sweet, bitter or bitter–sweet. Bitter–sweet sugar molecules, like certain artificial sweeteners such as saccharin, have both bitterness and sweetness as intrinsic features of their molecules[10]—neither response can be altered by exhaustive stepwise purification of such compounds[10]. Bitter–sweet sugar molecules appear to be 'polarised' on taste receptors[9] so that one end of the molecule binds to sweet receptor sites and the other to bitter receptor sites. It is not clear, however, whether these molecules distribute themselves, some on sweet receptors

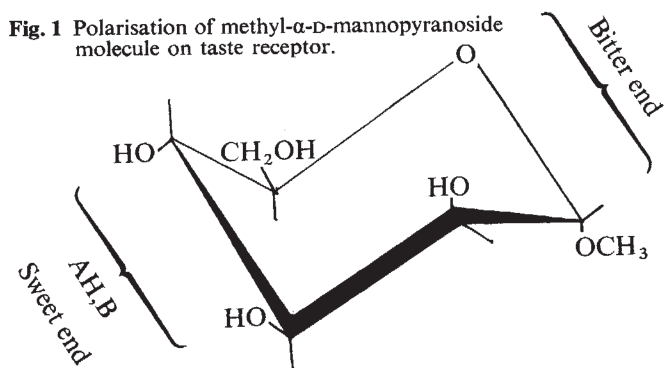

Fig. 1 Polarisation of methyl-α-D-mannopyranoside molecule on taste receptor.

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