LOWERING OF INNATE RESISTANCE OF THE LUNGS TO THE GROWTH OF BLOOD-BORNE CANCER CELLS IN STATES OF TOPICAL AND SYSTEMIC STRESS

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Summary.—The survival and clonogenic growth (measured in terms of colony forming efficiency (CFE)) of intravenously injected (i.v.) Walker (W256) tumour cells in the lungs of rats was greatly enhanced by states of topical and systemic stress induced by the intraperitoneal (i.p.) injection of rats with a single dose of $10^{-5}$-$10^{-3}$ mmol g$^{-1}$ body weight of adrenaline and other $\beta$-adrenergic agonists, inflammatory agents (including local x-irradiation), convulsive seizures, "tumbling" or physical restraint. Lowering of innate resistance of the host to growth of seeded tumour cells induced by states of topical and systemic stress, and by the addition of an excess of lethally irradiated (LI) tumour cells to i.v. injected intact tumour cells, were all potentiated by treatment of rats with aminophylline, an inhibitor of cyclic AMP phosphodiesterase. Enhancement of tumour growth by systemic stress was inhibited by bilateral total or medullary adrenalectomy and is attributed to the release and actions of endogenous adreno-medullary hormones. Alpha-adrenergic and most non-adrenergic agents administered in maximum tolerated doses did not significantly affect host resistance to tumour growth in the lungs.

These findings, correlated with measurements of cyclic AMP in the lungs of normal and stressed rats, suggest that changes in the resistance of the host to tumour growth involve changes in cyclic nucleotide metabolism in the target tissues (tumour bed); possible mechanisms of action of cyclic nucleotides in this respect are discussed.

Enhanced growth of intravascularly injected tumour cells has been shown to occur in injured tissues of organs of rats (Fisher and Fisher, 1959a, b, 1962; Robinson and Hoppe, 1962; Alexander and Altmeier, 1964). Clonogenic growth of i.v. injected tumour cells in the lungs and other organs of rats and mice is markedly enhanced by prior x-irradiation of the target organ (Withers and Milas, 1973; Brown, 1973; van den Brenk et al., 1973a; van den Brenk and Kelly, 1973, 1974). Chemically induced inflammatory reactions similarly enhance tumour colony forming efficiency (CFE) in the lungs of rats (van den Brenk et al., 1974). In these various investigations, suggestive but not clear-cut evidence was obtained that tumour growth was stimulated by systemic stress induced in the animal by tissue injury; it was essentially attributed to local pathophysiological changes produced in the target organ by the injurious agent. However, the studies of Fisher and Fisher (1959b) did indicate that systemic stress affected tumour growth since a laparotomy stimulated growth of tumour in the liver of rats. Recently, Peters (1975) has reported that laparotomy enhanced take and growth of subcutaneously injected tumour cells in mice. These findings suggest that systemic stress indirectly lowers an innate resistance of tissues to growth of cancer cells. Presumably this results from pharmacologically induced changes in the target tissue which are mediated via neuroendocrinal pathways. This common property of agents, which
cause topical or systemic stress, of lowering resistance of tissues to tumour growth under appropriate conditions has caused us to investigate the effects of $\beta$-adrenergic agents on CFE and the possibility that a common biochemical mechanism of action is involved which is fundamentally associated with the perturbation of cyclic nucleotide metabolism in the tumour bed. The release of adrenaline, its activation of $\beta$-adrenergic receptor bound adenyl cyclase (AC) and stimulation of cyclic adenosine-3'5'-monophosphate (c-AMP) in a variety of tissues, including lung (Robinson, Butcher and Sutherland, 1971; Jost and Rickenberg, 1971) plays a principal role in the physiological changes seen in states of stress. We have compared the effects on tumour CFE in the lungs of treating rats with $\beta$-adrenergic agonists with that of a variety of other pharmacologically active drugs, and similarly studied the effects on tumour growth of aminophylline, an inhibitor of phosphodiesterase (PD), the enzyme which causes metabolic degradation of c-AMP in tissues. The effects of chemically and physically induced systemic stress on tumour CFE and measurements of c-AMP levels in irradiated and unirradiated lungs of rats are described.

MATERIALS AND METHODS

The lung colony assay technique, using a subline of Walker (W256) tumour cells, prepared in single cell suspension and injected i.v. in SPF Caworth Farm strain rats, has been described previously (van den Brenk, Sharpington and Orton, 1973b). Tumour CFE was determined 8 days after the injection of tumour cells as, CFE = $N_L/N$ where $N_L$ is the number of lung tumour macrocolonies produced on the surfaces of the lungs of a rat by the i.v. injection of $N$ tumour cells. The effect on CFE of treating rats with a particular agent was measured as the ratio $R(N_L) = \text{CFE}(T)/\text{CFE}(C)$ for 2 groups of 6–8 treated (T) rats and 6–8 control (C) rats of the same age which had been injected i.v. with the same number of tumour cells from the same tumour cell suspension. The significance of $R$ was calculated by Student’s “t” test with Bessel’s correction. Since increases in the number of 8-day old tumour colonies per lung were shown to cause proportional increases in lung wet weight, a corresponding ratio $R(w)$ based on lung weight was calculated provided the treatment with the agent alone caused no significant change in lung weight of rats (measured per unit body weight) 8 days later. It was difficult to count accurately lung colonies in excess of 200 per rat; in such experiments $R(w)$ provided the better basis for comparisons of tumour growth. Since tumour CFE depends very greatly on age of rat, rats of approximately the same age (within a range of 5 days) were selected for each treated and corresponding control group. Each rat was weighed before it was injected with tumour cells and again when killed to measure tumour growth 8 days later.

Drugs and dosages

The following drugs were used: o-Acetylcholine chloride (Hopkin & Williams Ltd), adenosine-3',5'-monophosphate cyclic (Hopkin and Williams Ltd), angiotensin amide (Hypertensin, CIBA Laboratories Ltd.), atropine sulphate, bradykinin (BRS 640, Sandoz Ltd.), bretylium tosylate (Darenthin, Burroughs Wellcome and Co.), 2-bromolsergic acid diethylamide tartrate (BOL 148, Sandoz Ltd.), cyclophosphamide monohydrate (Endoxana, W. B. Pharmaceuticals Ltd), N6,02'-dibutyryl adenosine-3',5'-cyclic phosphoric acid sodium salt (d,b-cAMP, BDH Chemicals Ltd), glucagon (Eli Lilly & Co. Ltd), guanethidine sulphate (Ismelin, CIBA Laboratories Ltd), histamine acid phosphate (BDH Chemicals Ltd), 5-hydroxytryptamine creatinine sulphate (serotonin creatinine sulphate, BDH Chemicals Ltd), imidazole (BDH Chemicals Ltd), isopropyl noradrenaline sulphate (isoprenaline, Burroughs Wellcome & Co.), mepyramine maleate (Anthisan, May & Baker Ltd), N-2(3,4-methylenedioxyphenylisopropyl) noradrenaline (Protokylol, Lakeside Laboratories), N-methyl-noradrenaline (adrenaline, Hopkin & Williams Ltd), L-noradrenaline hydrochloride (L-arterenol, Sigma Chemical Co.), pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd), pentylenetetrazole (Leptazol, Martindale Samooore Ltd), phenoxybenzamine hydrochloride (Dibenzyl, Smith Kline & French Laboratories
Systemic rats
Six-week single adrenalectomy described pentobarbitone injected thorax before injection. Theophylline
agents (ICI Ltd), prostaglandins A, E₁, E₂, F₂α (Upjohn Ltd), sodium acetazolamide (Diamox, Lederle Laboratories Ltd), sodium ethacrynate (Edecrin, Merek Sharp and Dohme Ltd), theophylline ethylene diamine (aminophylline, Antigen Ltd), theophylline hydrate (BDH Chemicals Ltd, vasopressin (Pitressin, Parke-Davis & Co.). All other chemicals were analytical grade. ³H-thymidine was supplied by the Radiochemical Centre, Amersham, and Compound 48/80 was kindly donated by Wellcome Research Laboratories. Cellulose sulphate was prepared as described previously (van den Brenk et al., 1974).

Unless stated otherwise, the drugs were injected intraperitoneally (i.p.) in single doses, expressed in terms of mmol drug/g body weight. Each agent was tested for acute toxicity; the “maximum tolerated” dosage used in tumour injected rats did not exceed one-half of the LD₅₀ dose or 10⁻⁴ mmol drug/g body weight. Signs of prostration and stress occurred within a few min after injection of β-adrenergic and other agents in “maximum tolerated” dosages but the rats recovered relatively rapidly. Tumour CFE was measured only in those experiments in which at least 80% of rats had survived and gained weight at a mean rate of not less than 2 g/day for 8 days after the injection of the drug and the tumour cells.

Local x-irradiation of the thorax (LTI)
A single dose of 1000 rad to the whole thorax or hemithorax with the remainder of the body of the rat shielded was given under pentobarbitone sodium anaesthesia 7 days before injection of the tumour cells, as described previously (van den Brenk et al., 1973a).

Adrenalectomy
Bilateral total (TAₓ) or medullary (MAₓ) adrenalectomy was performed and replacement therapy given as described previously (van den Brenk et al., 1974).

Systemic stress
Techniques.—Three methods of stressing rats were used:

1. Chemical stress—convulsive seizures.—Six-week old rats were injected i.p. with a single dose of 1·2–12·5 mg pentylenetetrazole (PTZ), an agent which induces seizures in rats. The seizures are violent and sustained (grand mal) and prove fatal within 5–10 min in unanaesthetized rats when the dose of PTZ exceeds 5 mg; a lower dose of 3 mg PTZ causes slight twitchings (petit mal), a dose of 5 mg PTZ causes grand mal seizures from which approximately 80% of rats recover and survive. In rats which have been anaesthetized with 38 mg pentobarbitone sodium/kg body weight the injection of 5 mg PTZ causes an earlier awakening of the rats than usual but no seizures develop and all rats survive. An increase in the dose to 12·5 mg PTZ in anaesthetized rats causes rapid awakening and approximately 50% rats develop grand mal seizures and the remainder petit mal attacks, but there are no fatalities. Tumour CFE was measured under these various conditions of treatment with PTZ given 5–10 min after the i. v. injection of tumour cells.

2. Physical stress.—(a) Tumbler technique.—To cause stress, a group of rats was placed in a closed cylindrical drum which could be rapidly rotated about its central axis on a horizontal rod held clamped in a vice. The drum was fitted with a fixed internal vane which tumbled the rats inside the drum as it was spun by hand at a rate of about 2 rev/s changing from clockwise to anti-clockwise every 30 s. The stress of tumbling was supplemented by that of loud noise produced by an assistant hammering a metal rod; the noise intensity was not measured. The rats were tumbled for three 5-min periods separated by 2 rest periods of 1 h each. Tumour CFE was measured in rats which were tumbled 24 h before, 30 min after, or 24 h after i. v. injection of tumour cells. (b) Stress immobilization.—The technique of Selye (1954) was used to stress rats by tapping conscious rats to a wooden lathe with Sellotape for a total time of 3 h. The rats were taped head down on to the board which was held at an angle of about 80° to the horizontal plane. This procedure induced struggling but prevented escape. Immobilization was commenced 30 min after i.v. injection of intact or adrenalectomized rats with tumour cells.

Assay of adenosine-3',5'-monophosphate cyclic (c-AMP) in lungs
Each rat was anaesthetized with pentobarbitone sodium, the abdomen was widely opened and the aorta and inferior vena cava
were cut across to exsanguinate the rat. The anterior wall of the thorax was cut away and the lungs were rapidly removed and weighed. The inner hilar regions of each lung were resected and discarded. The remaining peripheral portions of each lung were added to 2 ml ice-cold 0·3 mol/l perchloric acid (PCA) in a pre-weighed glass pot and weighed. In the cold room the lung was finely minced with scissors, homogenized in a blender at 0°C and then centrifuged at 2500 g for 7 min. The supernatant was removed and 1 mol/l KH₂PO₄ was added to the supernatant to give a final concentration of 0·2 mol/l KH₂PO₄, and then the pH was adjusted to 5·5 by adding 5 mol/l KOH. After recentrifugation (2500 g for 10 min) the supernatant was removed and used to measure the concentration of c-AMP using a chemical test kit supplied by Boehringer Mannheim (Biochemica) for the radio-isotope dilution protein binding procedure described by Gilman (1970). Before the assay of c-AMP, most supernatants were stored at −70°C for 24–48 h. The pellet obtained from the PCA extract of lung homogenate was resuspended in 5% PCA and the desoxyribosenucleic acid (DNA) concentration was determined by the method of Schmidt and Thannhauser (1945) modified by Munro and Fleck (1966) as previously described (van den Brenk and Stone, 1972). The c-AMP concentration in lung tissue was calculated in terms of pmol c-AMP/mg DNA for individual rats. Two experiments were performed to determine the effect of local x-irradiation of the lungs on c-AMP levels: in the one experiment both lungs were irradiated in each rat and c-AMP concentrations compared with those in sham irradiated controls. In the other experiment, either the right or left lung was locally irradiated in equal numbers of rats in the group and c-AMP and DNA were measured in the irradiated and shielded lung tissue of each rat, after removing and discarding the post-caval lobe situated in the midline of the thorax. The c-AMP values for the 2 sides were compared to minimize any indirect (abscopal) effects of irradiation on concentrations of the nucleotide in lung tissue.

Specific activity of DNA in lung

This was measured in unirradiated and irradiated rats which were killed 2 h after i.p. injection with 50 μCi ³H-thymidine. The rats were exsanguinated, the lungs were removed and weighed and the DNA concentration and its specific activity in the peripheral lung tissues were measured as described previously (van den Brenk et al., 1975).

RESULTS

Stimulation of tumour CFE in lung by β-adrenergic agonists

A single i.p. injection of 10⁻⁵–10⁻⁴ mmol adrenaline or isoprenaline (ISOP)/g body weight caused marked increases in CFE in the lungs when the adrenergic agent was injected from 2 h before to 2 h after i.v. injection of the tumour cells (Table I). The β-adrenergic drug, protokylol, stimulated CFE also. Tumour CFE is high in weanling rats and decreases rapidly with increase in age (van den Brenk, Sharpton and Orton, 1973b). Beta-adrenergic drugs stimulated tumour CFE in rats of all ages, the effect increasing with increase in age of host. The rapid decrease in tumour CFE which occurs with increase in age of untreated rats is accompanied by a decrease in tumour growth rate; the tumour colonies become smaller and this contributes to the smaller increases in lung weight produced by the growth of tumour cells in older rats. Besides increasing CFE, adrenaline and ISOP caused the tumour cells to grow to larger-sized colonies, which was mainly responsible for the increases in lung weight produced by the agents in older rats.

Maximum tolerated single doses of a variety of agonists which differed in their pharmacological actions and tissue specificities which were injected 30 min after i.v. injection of tumour cells had no significant effect on tumour CFE. Examples of negative results obtained with the α-adrenergic agent, noradrenaline, and various other agents are shown in Table I. Negative results (not tabulated) were also obtained with a variety of antihistamines, and the diuretic agents, mannotol, edecrin, NH₄Cl, CaCl₂ and acetazolamide injected in doses of 10⁻⁴–10⁻³ mmol drug/g body weight. The prosta-
Table I.—Effects on Tumour CFE in the Lungs of a Single i.p. Injection of Adrenergic and Other Agents (dose expressed in mmol/g body weight unless stated otherwise) given 30 min after i.v. Injection of Rats with W256 Tumour Cells. Ratios R(NL) and R(w) Calculated for Number of Tumour Colonies (NL) and Lung Weight (w) Respectively for each Two Groups of Treated and Control Rats* (6–8 Rats per Group) and Significances (s)†

| Age of rats (weeks) | Dose of agent(s) | R(NL)  | R(w)  |
|---------------------|------------------|--------|-------|
| 10⁴                 | 5                | 5 × 10⁻⁴ Adrenaline | 0·82   | 1·04  |
| 2 × 10³             | 4                | 10⁻⁴ Adrenaline | 2·95 (s) | 1·22 (s) |
| 10³                 | 5                | 10⁻⁴ Adrenaline | 1·30   | 1·18  |
| 10⁴                 | 5                | 10⁻⁴ Adrenaline | 2·45 (s) | 1·37 (s) |
| 10⁴                 | 4                | 10⁻⁴ Adrenaline | 7·78 (s) | 2·48 (s) |
| 10⁴                 | 6                | 10⁻⁴ Adrenaline | 3·11 (s) | 1·93 (s) |
| 2 × 10³             | 4                | 10⁻⁴ Isoprenaline | 1·22   | 1·06  |
| 10⁴                 | 8                | 5 × 10⁻⁴ Isoprenaline | 7·73 (s) | 1·20 (s) |
| 10³                 | 4                | 10⁻⁴ Isoprenaline | 4·30 (s) | 1·59 (s) |
| 10⁴                 | 4                | 10⁻⁴ Isoprenaline | 4·27 (s) | 1·71 (s) |
| 10⁴                 | 8                | 10⁻⁴ Isoprenaline | 1·29 (s) | 1·38 (s) |
| 10⁴                 | 10               | 10⁻⁴ Isoprenaline | 16·75 (s) | 1·01  |
| 10⁴                 | 10               | 10⁻⁴ Isoprenaline | 8·00 (s) | 1·02  |
| 10⁴                 | 6                | 10⁻⁴ Protokylol | 10·02 (s) | 1·20  |
| 2 × 10³             | 4                | 10⁻⁴ L-Noradrenaline | 1·66   | 1·10  |
| 10³                 | 4                | 50 µg Angiotsensin amide | 1·87   | 1·48  |
| 10³                 | 7                | 0·2 mg Vasopressin (IVI) | 1·40   | —     |
| 10³                 | 7                | 0·4 mg Vasopressin (IMI) | 1·73   | —     |
| 10³                 | 4                | 10⁻⁴ Atropine | 0·95   | 0·99  |
| 10³                 | 5                | 10⁻⁴ o-Acetylcholine | 1·16   | 1·08  |
| 10³                 | 5                | 10⁻⁴ Histamine | 0·95   | 1·13  |
| 10³                 | 5                | 10⁻⁴ 5-Hydroxytryptamine | 2·23   | 1·42 (s) |
| 10³                 | 5                | 10⁻⁴ 2-Bromolyseryge acid | 0·81   | 1·02  |
| 10³                 | 5                | 2 mg kg⁻¹ Guanethidine sulphate | 0·39 (s) | 0·94 |
| 10³                 | 5                | 2 mg kg⁻¹ Bretylum tosylate | 0·30 (s) | 0·94 |
| 10³                 | 5                | 10 mg kg⁻¹ Cellulose sulphate | 3·22 (s) | 1·52 (s) |
| 10³                 | 5                | 2 mg kg⁻¹ Guanethidine plus† | 5·00 (s) | 1·79 (s) |
| 10³                 | 5                | 10 mg kg⁻¹ cellulose sulphate | 0·50 (s) | 1·00 |
| 10³                 | 5                | 2 mg kg⁻¹ Bretylum plus† | 1·30   | 1·18  |

* Control rats injected i.p. with 0·2 ml isotonic saline.
(s) Signifies P < 0·001 to < 0·05.
† Guanethidine sulphate and bretylum tosylate injected i.p. 10 min before CS or adrenaline.

Glandins PGA, PGE₁, PGE₂ and PGF₂α, histamine and bradykinin failed to increase CFE significantly. I.p. injection of 10⁻⁴ mmol 5-hydroxytryptamine/g body weight, which caused marked prostration of rats, stimulated CFE (Table I, see below). Glucagon injected 30 min to 24 h after i.v. injection of the tumour did not affect CFE.

Effects of aminophylline on CFE

I.p. injection of rats with single doses of the phosphodiesterase inhibitor, theophylline ethylene diamine (aminophylline), did not stimulate CFE but 2–3 doses of 10⁻⁴ mmol aminophylline/g body weight, injected from 30 min before to 4 h after injection of the tumour cells caused modest but variable increases in CFE (Table II). Stimulation of CFE in rats treated with adrenaline or ISOP was markedly enhanced by additional treatment with aminophylline (Table III, Fig. 1). The combined effect of ISOP and aminophylline of stimulating CFE was confined to a period from a few hours before to 4 to 6 h after i.v. injection of the tumour cells; CFE was not significantly affected.
TABLE II.—Effect of Treatment of Rats with Aminophylline on Clonogenic Growth in the Lungs of i.v. Injected Tumour Cells. One to Three Doses of Aminophylline (mmol/g body weight) were Injected Intraperitoneally 30 min before to 4 h after i.v. Injection of Groups of 6–8 Rats with $10^4$ W256 Tumour Cells

| Age of rats (weeks) | Dose of aminophylline (no. of doses) | Time(s) | R(NL)* |
|---------------------|-------------------------------------|---------|---------|
| 4                   | $10^{-4}$ (1)                       | −30 min | 1.64    |
| 4                   | $10^{-4}$ (1)                       | +30 min | 3.00 (s) |
| 5                   | $10^{-4}$ (1)                       | −30 min | 0.80    |
| 5                   | $10^{-4}$ (1)                       | +30 min | 1.08    |
| 5                   | $10^{-4}$ (1)                       | +2 h    | 1.33    |
| 5                   | $10^{-4}$ (1)                       | +4 h    | 1.76    |
| 5                   | $10^{-4}$ (2)                       | −30 min | 2.62 (s) |
| 5                   | $10^{-4}$ (2)                       | +30 min | 0.77    |
| 5                   | $10^{-4}$ (3)                       | +2 h    | 3.64 (s) |
| 5                   | $10^{-4}$ (3)                       | +4 h    | 1.37    |
| 7                   | $10^{-4}$ (1)                       | −30 min | 1.33    |
| 7                   | $10^{-4}$ (2)                       | −30 min | 1.37    |
| 7                   | $10^{-4}$ (3)                       | +2 h    | 2.91 (s) |
| 7                   | $10^{-4}$ (3)                       | +4 h    | 1.75    |
| 8                   | $10^{-5}$ (1)                       | +30 min | 1.73    |
| 8                   | $3.3 \times 10^{-5}$ (1)            | +30 min | 1.77    |
| 8                   | $10^{-4}$ (1)                       | +30 min | 1.96    |
| 10                  | $10^{-4}$ (1)                       | −30 min | 2.25    |
| 10                  | $10^{-4}$ (1)                       | +30 min | 1.75    |

* See Table I.

when the rats were treated with the 2 agents 24 h before or 24 h after injection of the tumour cells (Fig. 2). The combined effect of ISOP and aminophylline of stimulating tumour CFE was comparable with that of injecting an excess of lethally irradiated (LI) tumour cells together with living tumour cells ("Révész Effect") but was not as effective as that obtained when the lungs of rats were locally irradiated (LTI) with 1000 rad 7 days before the i.v. injection of tumour cells (Table III). Combined treatment of rats with ISOP and aminophylline significantly enhanced the effects of LI cells and LTI of stimulating CFE in young (3–4-week old) but not in older animals (results not tabulated).

Effects of systemic stress on tumour CFE

Severe seizures induced in rats by an i.p. injection of pentylenetetrazole (PTZ) given 5–10 min after i.v. injection of W256 cells significantly increased tumour CFE; this effect was abolished when the rats had been anaesthetized to prevent convulsions (Table IV). It is noteworthy that anaesthesia did not similarly affect stimulation of tumour CFE by the injection of $\beta$-adrenergic agents and aminophylline.
Fig. 2.—Effect of 10^{-4} mmol isoprenaline combined with 10^{-4} mmol aminophylline/g body weight, injected together i.p. at various intervals before or after i.v. injection of 5-week old male rats with 10^4 W256 cells, on the number of tumour macrocolonies produced in the lungs. Six to 8 rats per point: ISOP plus aminophylline (○), controls (●).

(Table V), nor did anaesthesia reduce stimulation of CFE by LTI or LI cells or by inflammatory agents such as cellulose sulphate and Compound 48/80 (results not tabulated). These findings suggested that stimulation of CFE by the chemical convulsant in the rat was an indirect effect due to systemic stress, and that it was probably mediated by the release of adrenal hormones. This conclusion was supported by our finding that physically induced systemic stress (“tumbling” of rats and restraint) similarly stimulated tumour CFE in rats, and that bilateral total (TAx) or medullary (MAx) adrenalectomy abolished this effect of physical stress of stimulating CFE (Table VI). Systemic stress did not stimulate CFE if the rats were stressed 24 h before or 24 h after the i.v. injection of tumour cells. Since MAx was as effective as TAx in inhibiting tumour growth in stressed rats, we conclude that the acute release of endogenous adrenaline from the adrenals in states of stress plays a major role in stimulating tumour growth. It is noteworthy that adrenalectomy consistently caused modest but not statistically significant reductions in tumour CFE in unstressed rats and also slightly reduced the effects of LTI on CFE (Table VI, van den Brenk et al., 1973). However, TAx and MAx did not significantly reduce stimulation of tumour growth by topical stressors, namely LTI, inflammatory agents or the injection of a β-adrenergic agent (results not tabulated).

5-Hydroxytryptamine (5OH-T)

In intact rats tumour CFE was stimulated by large doses of 5OH-T, a chemical mediator of inflammation, but not in rats in which MAx had been previously per-
**Table III.**—Effect of Treatment of Rats with Isoprenaline (ISOP) combined with Aminophylline on CFE of I.v. Injected W256 Tumour Cells (6 Rats used in each Test and corresponding Control Group). The two Compounds were Injected Intraperitoneally as Single Doses 1 h after i.v. Injection of Tumour Cells in A and C; in B, the two agents were Injected 30 min before, and the same Treatment Repeated 4 h after i.v. Injection of Tumour Cells

| N   | Age of rats (weeks) | Dose of agent(s)                      | R(NL) | P    | R(wl) | P    |
|-----|---------------------|--------------------------------------|-------|------|-------|------|
| A   |                     |                                      |       |      |       |      |
| 10^4 | 8                   | 10^-4 ISOP + 10^-4 aminophylline      | 9.62  | <0.01| 1.24  | <0.05|
| 10^4 | 4                   | 10^-4 ISOP + 10^-4 aminophylline      | 9.05  | <0.001| 3.58  | <0.001|
| 10^4 | 4                   | 10^-4 ISOP + 10^-4 aminophylline      | 5.70  | <0.001| 1.87  | <0.01|
| 10^3 | 7                   | 10^-4 ISOP + 10^-4 aminophylline      | 19.33 | <0.001| 1.10  | n.s. |
| 10^4 | 8                   | 10^-4 ISOP + 10^-4 aminophylline      | 16.65 | <0.001| 1.67  | <0.01|
| 10^4 | 10                  | 10^-4 ISOP + 10^-4 aminophylline      | 19.25 | <0.001| 1.07  | n.s. |
| B   |                     |                                      |       |      |       |      |
| 10^4 | 6                   | (a) 10^-4 ISOP + 10^-4 aminophylline  | 16.60 | <0.001| 2.01  | <0.01|
|      | 6                   | (b) (2 x 10^6 Li cells added only)    | 15.50 | <0.001| 1.43  | <0.01|
|      | 6                   | (c) (2 x 10^6 Li cells added; 10^-4 ISOP + 10^-4 aminophylline) | 38.24 | <0.001| 2.54  | <0.001|
|      |                     | Difference (b) and (c)               |       | <0.001|       |      |
| C   |                     |                                      |       |      |       |      |
| 10^4 | 7                   | (a) 1000 rad LTI (Day -7)             | 47.00 | <0.001| 1.34  | <0.001|
| 10^4 | 7                   | (b) 10^-4 ISOP, 10^-4 aminophylline,  |       |      |       |      |
|      |                     | 1000 rad LTI (Day -7)                 | 79.00 | <0.001| 1.37  | <0.001|
|      |                     | Difference (a) and (b)               |       | <0.05 | n.s.  |      |

In B: 2 x 10^6 W256 cells, which had been lethally irradiated (10 K rad x-rays) in vitro were added to the i.v. injected 10^4 intact W256 cells in (b) and (c).

In C: the lungs were locally irradiated with a single dose 1000 rad x-rays 7 days before i.v. injection of the rats with 10^4 W256 cells in both (a) and (b); the drugs were injected 1 h after the i.v. injection of cells in (b) only.

formed (Tables I, VI). Consequently, stimulation of CFE by 5OH-T is largely attributed to an indirect stressor effect (adrenergic stimulation) of the drug. Similarly, drug induced stress may account for the modest increases in tumour CFE which were produced by other pharmacologically active drugs such as angiotensin amide and vasopressin (Table I) and PGE_1, when large doses were given which caused acute prostration. An experiment was also performed in which rats were maintained either at 4°C or at 37°C for 8 h after i. v. injection of tumour cells to induce a mild but more prolonged form of stress, but the treatments did not significantly affect tumour CFE (results not tabulated).

**Sympatheticiolytic agents**

Injection of rats with guanethidine sulphate or bretylium tosylate caused significant reductions in tumour CFE; these agents inhibited the effect of adrenaline of stimulating CFE also, but not that of the inflammatory agent, cellulose sulphate (Table I). Bretylium tosylate did not reduce stimulation of tumour CFE by LTI (results not tabulated).

**α- and β-Adrenergic blocking drugs**

Several experiments were performed to measure the effects of drugs which block α- and β-adrenergic receptors on tumour CFE in the lungs of rats. Results of one of these experiments in 4-week old rats are shown in Table VII, in which dibenzyline (α-blocker) and propranolol (β-blocker) were used singly or in combination with the β-agonists, adrenaline and ISOP, respectively. Injected in large doses, dibenzyline and propranolol stimulated CFE but did not significantly decrease stimulation of CFE by β-agonists. Experiments in 6- to 10-week old rats gave more variable but similar results,
TABLE IV.—Effect of Intraperitoneally Injecting Unanaesthetized and Anaesthetized 6-week Old Female Rats with Pentyline Tetrazole (PTZ)* on the Number of Lung Tumour Colonies (N_L) Produced by Intravenously Injecting 10³ W256 Tumour Cells (Groups 1–7) or 10⁴ W256 Cells (Groups 8, 9). Eight Rats per Group

| Group (treatment) | N_L     |     |
|-------------------|---------|-----|
| 1. (Saline)       | 10±2    |     |
| 2. (Anaesthetic)  | 6±2     |     |
| 3. (1·2 mg PTZ)   | 11±4    |     |
| 4. (2·5 mg PTZ)   | 9±3     |     |
| 5. (5 mg PTZ)     | 40±13   |     |
| 6. (Anaesthetic plus 5 mg PTZ) | 6±2 | (P<0·01) |
| 7. (Anaesthetic plus 12·5 mg PTZ) | 20±7 | (P<0·02) |
| 8. (Saline)       | 189±41  |     |
| 9. (5 mg PTZ)     | 299±25  |     |

* Rats were injected with PTZ 5·10 min after i.v. injection of tumour cells. Rats in Groups 2, 6 and 7 were anaesthetized with 38 mg pentobarbitone sodium/kg body weight injected intraperitoneally 15·10 min before the tumour cells. The dose of 5·10 mg PTZ produced one death from convulsions in Groups 5 and 9; the other 7 rats in Group 5 also convulsed. Doses of 1·25 and 2·5·10 mg PTZ caused no convulsions or deaths; 5·10 mg PTZ wakened most anaesthetized rats within 10·10 min without causing convulsions; 12·5·10 mg PTZ wakened anaesthetized rats within a few min, 3 rats convulsed severely, but all 8 rats in the group recovered. This dose (12·5·10 mg PTZ) always caused death in unanaesthetized rats from convulsions within 5·10 min.

TABLE V.—Effect of Anaesthesia* on Stimulation of Tumour CFE caused by the i.p. Injection of 4-week old Female Rats with 10⁻⁵ mmol Adrenaline or 10⁻⁴ mmol ISOP plus 10⁻⁴ mmol Aminophylline/g body weight 30·10 min after i.v. Injection of 10⁴ W256 Cells; 6 Rats per Group

| Treatment (anaesthetized ±) | No. of lung colonies (±s.e.) | Lung weight (g) (±s.e.) |
|-----------------------------|-----------------------------|------------------------|
| Nil (–)                     | 81±19                      | 1·07±0·03              |
| Nil (+)                     | 139±33                     | 1·17±0·07              |
| 10⁻⁵ Adrenaline (–)         | 630±73                     | 2·66±0·42              |
| 10⁻⁵ Adrenaline (+)         | 530±68                     | 2·24±0·33              |
| 10⁻⁴ ISOP plus 10⁻⁴ amino-  | 733±37                     | 3·83±0·23              |
|  | phylline (–)               |                           |                       |
| 10⁻⁴ ISOP plus 10⁻⁴ amino-  | 667±40                     | 2·86±0·32              |
|  |  | phylline (+)               |                           |                       |

* 38 mg pentobarbitone sodium IPI 15·10 min before i.v. injection of tumour.

that is to say, the β-blockers, propranolol and practolol, caused modest increases in CFE and caused slight but not significant reductions in CFE in rats treated with β-agonists.

Sodium fluoride, imidazole, cyclic AMP

I.p. injection of rats with single doses of 10⁻⁵ mmol NaF/g body weight caused a significant reduction in tumour CFE, (R(N_L) = 0·32), but had no significant effect on stimulation of CFE by ISOP. In these experiments, NaF and ISOP, injected singly or together, did not affect body growth rates, which averaged 4·9–5·2 g/day from the day of injection of drugs and tumour cells till the rats were killed to count tumour colonies 8 days later. I.p. injection of rats with 2 doses of 10⁻⁴ mmol imidazole/g body weight 10·10 min before and 3·10 h after i.v. injection of rats with W256 cells had no significant effect on tumour CFE. Incubation of W256 cells with 10⁻⁴ mol/l imidazole for 1·10 h at 37°C in vitro, as previously described (van den Brench et al., 1974), did not significantly affect CFE, nor did incubation of W256 cells with 10⁻⁵ mol/l dibutyrly cyclic AMP affect CFE. I.v. injection of rats with 10⁻⁶ mmol dibutyrly cyclic AMP or 10⁻⁵ mmol c-AMP/g body weight 30·10 min after i.v. injection of W256 cells did not affect tumour CFE (results not tabulated).

Effects of age, local x-irradiation and β-adrenergic agonists on cyclic AMP in lungs

The concentration of c-AMP in lung tissue of intact rats decreased with age; in 3 groups of 5–7 female rats killed at 4, 7 and 10 weeks of age the mean concentrations were 77 ± 4, 60 ± 10 and 33 ± 3 pmol c-AMP/mg DNA respectively. Local x-irradiation of the thorax in 10-week old rats with a single dose of 1000 rad caused an initial rapid decrease in c-AMP in lung; this was followed by recovery to values which were about 30% in excess of normal at 3–7 days,
Table VI.—Effects of Physical Stress *and Injection of 10⁻⁴ mmol 5-hydroxytryptamine/ g Body Weight on Tumour CFE in Intact and Adrenalectomized Rats Injected Intravenously with 10⁴ W256 Cells (6–8 Rats per Group). Mean Gain in Body Weight of Rats (ΔWg) Shown for the 8 Days Elapsing from the Day of Injection of Tumour Cells until the Day of Sacrifice; TAₓ, Bilateral Total Adrenalectomy, MAₓ, Bilateral Medullary Adrenalectomy

| Stress (TAₓ, MAₓ) | Adrenaline | ΔW(g) (±s.e.) | N_L (±s.e.) |
|-------------------|------------|---------------|-------------|
| A. Nil (5-week old rats) | | 49 ± 2.2 | 106 ± 32 |
| | Stressed 24 h before VI cells | - | 48 ± 0.6 | 97 ± 28 |
| | Stressed 30 min after VI cells | - | 47 ± 1.0 | 255 ± 55 (s) |
| | Stressed 24 h after VI cells | - | 46 ± 2.0 | 90 ± 2.0 |
| B. Nil (7-week old rats) | | 25 ± 2 | 2 ± 0.8 |
| | Stressed after VI cells | TAₓ | 24 ± 2 | 53 ± 12 |
| | Nil | TAₓ | 8 ± 2 | 0.3 ± 0.3 |
| | Stressed after VI cells | TAₓ | 9 ± 2 | 2 ± 1.7 |
| | Nil | MAₓ | 29 ± 1 | 2 ± 1.8 |
| | Stressed after VI cells | MAₓ | 24 ± 1 | 4 ± 2.5 |
| C. Nil (6-week old rats) | | 33 ± 3 | 17 ± 8 |
| 10⁻⁴ 5-hydroxytryptamine | 10⁻⁴ 5-hydroxytryptamine | | 40 ± 4 | 165 ± 63 (s) |
| | | MAₓ | 38 ± 3 | 11 ± 4 |

* In A, groups of 6 rats were stressed by "tumbling" in a drum; in B, the rats were stressed by "restraint" (see Materials and Methods); in C, 5-hydroxytryptamine was injected i.p. 1 h after i.v. injection of the tumour. TAₓ and MAₓ were performed 24 h before injection of the tumour cells. After TAₓ, maintenance therapy with cortisone was delayed for 24 h after i.v. injection of tumour cells.

Table VII.—Effect of 10⁻⁴ mmol Dibenzyline and 10⁻⁴ mmol Propranolol g⁻¹ Injected Intraperitoneally 15 min after i.v. Injection of 4-week old Female Rats with 10⁴ W256 Cells on the number (N_L) of Lung Tumour Macrotumours counted 8 Days later, and of the Effect of Pretreatment with these Agents on Stimulation of CFE by 10⁻⁵ mmol Adrenaline or Isoprenaline g⁻¹ injected 30 min after i.v. Injection of the Tumour Cells* (6 Rats per Group)

| Treatment | Lung weight N_L (g) |
|-----------|---------------------|
| I | Isotonic saline 81 ± 19 1.07 ± 0.03 |
| II | 10⁻⁵ adrenaline 630 ± 73 2.66 ± 0.42 |
| III | 10⁻⁴ dibenzyline 450 ± 106 2.19 ± 0.44 |
| IV | 10⁻⁴ propranolol 186 ± 32 1.26 ± 0.42 |
| V | 10⁻⁴ propranolol 199 ± 65 1.58 ± 0.38 |
| VI | 10⁻⁴ adrenaline 10⁻⁴ dibenzyline 356 ± 76 1.98 ± 0.54 |
| VII | 10⁻⁴ dibenzyline 424 ± 111 1.85 ± 0.39 |
| VIII | 10⁻⁴ propranolol 10⁻⁴ adrenaline 10⁻⁴ isoprenaline 402 ± 66 1.87 ± 0.30 |
| IX | 10⁻⁴ propranolol 298 ± 49 1.71 ± 0.39 |
| X | 10⁻⁴ isoprenaline |

* Adrenergic blockers (propranolol and dibenzyline) injected singly or in combination 15 min after i.v. tumour cells, followed by adrenaline or isoprenaline 15 min later, in Groups V, VI, VII and IX.

Table VIII.—Cyclic AMP Concentrations in Lung Produced 30 min after an Intraperitoneal Injection of 10⁻⁵ mmol Isoprenaline plus 10⁻⁴ mmol Aminophylline g⁻¹ Body Weight in Unirradiated Rats and in Rats given 1000 rad Local Thoracic Irradiation (LTI) 7 Days Previously

| Treatment | I | II |
|-----------|---|---|
| A. Nil | 60 ± 10 | 64 ± 11 |
| B. LTI | 191 ± 23 | 114 ± 32 |
| C. ISOP, aminophylline | 844 ± 55 | 1167 ± 327 |
| D. LTI, ISOP, aminophylline | 320 ± 44 | 861 ± 58 |

In experiment I, both lungs were irradiated in Groups B and D; in II, the right or left hemithorax was irradiated only in B and D, and c-AMP levels for the corresponding contralateral unirradiated (shielded) lungs in these rats are shown in A and C (5–6 seven-week old female rats per group). and a return to normal 10 days after LTI (Fig. 3). In 7-week old rats LTI caused more marked increases in c-AMP 7 days after lung irradiation (Table VIII). This effect was confined to the irradiated portions of the lungs. The cause of changes produced by irradiation in c-AMP levels in lung is not known, but the pattern of
initial decrease followed by recovery to increased levels at 3–7 days, and thereafter a return to normal values, resembles the pattern previously obtained for stimulation of tumour CFE in the lungs of rats by LTI (van den Brenk et al., 1973a). A compensatory hyperplastic reaction occurs in the lung after 1000 rad LTI; this causes a marked rise in the specific activity of the DNA which peaks at about 7 days (Fig. 4). The 2 sets of data in Fig. 3 and 4 indicate that reactive increases in cell proliferative activity and c-AMP synthesis, which are induced in the lung by x-ray damage, follow a rather similar time-course and can occur concurrently. A single dose of $10^{-4}$ mmol ISOP caused 14- to 19-fold increases in c-AMP in the lungs of rats killed 15 min after injection of the agonist. In irradiated lungs the $\beta$-agonist was less effective in this respect.

**DISCUSSION**

The majority of i.v. injected allogeneic or syngeneic tumour cells or tumour cell aggregates which seed in the lungs and other tissues of rats and mice fail to survive, replicate and produce tumour colonies (Williams and Till, 1966; Hill and Bush, 1969; Withers and Milas, 1973; Brown, 1973; van den Brenk et al. 1973b; Thompson, 1974). By injecting rats with W256 tumour cells which have been labelled in vitro with 5-($^{125}$I)iodo-2'-deoxyuridine or with tritiated thymidine, it has been shown that the majority of tumour cells trap in the lungs but disappear rapidly within the first 6–12h after injection (van den Brenk et al., 1975). The proportion of the injected tumour cells which survive and produce lung colonies is much higher in 3– to 4-week old weanling rats than in older recipients (van den Brenk et al. 1973b), but this developmental decrease in tumour CFE with age can be counteracted by inflammatory reactions induced in the tumour bed in the lungs by x-ray or other injury (van den Brenk et al., 1973a, 1974). Earlier studies (Fisher and Fisher, 1959a, b, 1960, 1962; Robinson and Hoppe, 1962; Alexander and Altmeier, 1964) had shown that growth of tumour cells transplanted in the liver and other tissues of the rat was increased by damage to the target tissues caused by contusion or the injection of nitrogen mustard; also, laparotomy stimulated tumour growth. In these experiments it was uncertain whether the effect of injury was topical and directly due to damage to the target tissues, or indirect and caused by systemic stress. Nevertheless, these various observations strongly suggest that tissues of organs which are physiologically intact exhibit considerable
natural resistance to the survival and growth of a primary challenge of transplanted cancer cells. Such resistance increases with age of host, and in view of the rapidity of its manifestation appears to be passive rather than active in nature. It cannot be reasonably attributed to the mounting of a specific immunological reaction to the tumour by the host, even if the transplanted cells are allogeneic and immunogenic. Furthermore, in the rat

this form of innate resistance acts independently of immunity since CFE of i.v. injected allogeneic tumour cells can be increased by local irradiation of the lungs in an immunized host (van den Brenk et al., 1973a), and since irradiation of mice very markedly increased survival and growth of transplanted tumour cells which were essentially non-immunogenic (Hewitt, Blake and Porter, 1973).

In this paper we have shown that innate resistance of host tissues to tumour growth can be greatly reduced by strong stimulation of β-adrenergic reactions: directly, by injection of rats with large doses of β-adrenergic agonists or indirectly, by the liberation of endogenous adreno-medullary adrenaline in a state of systemic stress induced in differing ways. Tumour CFE was not similarly affected by maximum tolerated doses of a variety of drugs which interact with other types of receptors and cause different pharmacological effects (Table I), nor by various endogenous mediators of inflammation (van den Brenk et al., 1974). It is significant that whereas lung tissue is richly endowed with β-adrenergic receptors, it lacks receptors to most of the other agonists which did not stimulate tumour growth in the lungs. An allosteric interaction between an agonist and its corresponding receptor, bound to adenyl cyclase (AC) usually causes an increase in the concentration of the intracellular "second messenger", adenosine-3'-5'-monophosphate cyclic (c-AMP), which is synthesized when AC is activated in the presence of complement (Sutherland and Rall, 1957; Robinson et al., 1971). Cyclic AMP is cleaved to AMP-5' by intracellular phosphodiesterase (PD); consequently, theophylline and its derivatives which inhibit PD increase the effects of β-agonists on tissue c-AMP. Adrenaline and ISOP markedly increase c-AMP concentrations in the lungs (Table VIII); prostaglandin E₁ causes a more modest increase in lung c-AMP (Jost and Rickenberg, 1971). During postnatal development the c-AMP concentration in various tissues decreases, whereas PD
activity increases (Forn et al., 1970); c-AMP in the lung of rats similarly increases with age (see above). We have been impressed by the correlation between the rise in tumour CFE and the increase in c-AMP concentration which occurs in lungs of rats following local x-irradiation and β-adrenergic stimulation, as well as by the potentiation of these changes by treatment of rats with a PD inhibitor, and by the corresponding effects of age and inflammatory agents on these parameters. Perturbations of c-AMP metabolism accompany inflammatory reactions in tissues (Bourne et al., 1974) which are growth stimulating (Menkin, 1961). An inflammatory response induced in the lungs of mice by a bacterial infection has been shown to cause a corresponding rise in c-AMP in these tissues, which seems to involve a malfunction at the β-adrenergic receptor level (Klein, Cory and Fisher, 1974). Stimulation of β-adrenergic receptors in salivary tissues in rats by ISOP causes marked rises in the salivary c-AMP concentration (Malamud, 1969) and induces rapid mitogenesis and sialadenotrophy which is due to proliferative, combined with hypertrophic, growth of salivary acinar cells (Selye, Veilleux and Cantin, 1961). These correlations between c-AMP levels and stimulation of growth of normal tissues in vivo caused us to speculate that agents which increase the c-AMP concentrations in a target organ also stimulate survival and growth of seeded tumour cells, and that changes in innate resistance of tissues to tumour growth in vivo are basically caused by perturbations in cyclic nucleotide metabolism.

We have postulated previously that stimulation of tumour CFE by inflammation of the tumour bed is due to the local release of trophic hormone(s), (growth stimulating substances, GSS), which are concerned in stimulating replicative growth of the normal tissues in the repair process associated with inflammation, and likewise that of seeded neoplastic cells (van den Brenk and Sharpton, 1971; van den Brenk et al., 1973a). The secretion of most hormones is a uni-directional event in which c-AMP functions as the second intracellular messenger (Robinson et al., 1971). Perturbations of cell metabolism caused in states of topical and systemic stress, in which β-adrenergic receptors are stimulated and c-AMP synthesis increased, are conceivably associated with trophic reactions of a protective reparative nature, mediated by secretion of GSS. The sialadenotrophic effect of ISOP in rats provides an example of the growth promoting actions of β-adrenergic stimulation. In the case of the polypeptide fibroblast growth factor (FGF), the growth stimulating effect on normal tissue fibroblasts in vitro (Gospodarowicz, 1974) has been shown to be due to the stimulation of guanosine 3′-5′-monophosphate cyclic (c-GMP) synthesis in “resting” (Go) cells. This nucleotide, with hydrocortisone and Ca++ as co-factors, is self-sufficient in acting as a mitogenic trigger which programmes fibroblasts for DNA synthesis and replication in the absence of serum (Rudland, Gospodarowicz and Seifert, 1974). Similarly, various other mitogenic substances which programme cells for replicative growth stimulate synthesis of c-GMP as a prelude to DNA synthesis (Abell and Monahan, 1973). In vitro, GSS present in serum also stimulates growth of neoplastic cells. The autonomous growth pattern of neoplastic (transformed) cells appears to be associated with an enzymatic imbalance which endows the cell with a capacity to generate its own growth signal. Preparing tumour cells in suspension for transplantation in vivo and their bathing in blood and tissue fluids would tend to remove GSS originally present in their micro-environment. This would make the supply of GSS by normal tissues at the site of seeding important in stimulating growth of the tumour cells before they become established and are able to condition their own micro-environment with GSS. Evidence of the importance of adequate concentrations of GSS for stimulating survival and clonogenic
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growth of normal and neoplastic mammalian cells is provided not only by serum requirements in vitro but by the use of conditioned media and “feeder layers” (Puck and Marcus, 1956), and in vivo by the effect of lethally irradiated (LI) cells (Révész, 1958), phenomena which depend on the elaboration and release of GSS by metabolically active cells.

Although increases in c-GMP precede replicative cell growth, increases in intracellular c-AMP in vitro and in vivo generally occur when replicative growth ceases spontaneously or is inhibited (Abell and Monahan, 1973) and so-called pleiotypic effects are induced, consisting of decreases in the rates of RNA, protein and DNA synthesis and a stimulation of protein degradation (Kolata, 1973). These changes affect both bi-directionally and unidirectionally controlled physiological systems (Goldberg et al., 1972) stimulate functions such as contraction, storage of cellular products and secretion, and cause a modulation of cellular activity from a state of growth towards that of contact-inhibition and cyto-differentiation. Thus, in skin c-AMP levels fluctuate with a pronounced diurnal rhythm, reaching a maximum during the resting phase of growth (Marks and Grimm, 1972). In vitro, c-AMP, dibutylryl cyclic AMP and ISOP also inhibit DNA synthesis in PHA- and concanavalin A stimulated lymphocytes (Abell, Kamp and Johnson, 1970; Johnson and Abell, 1970; Krug et al., 1972) and the growth of HeLa and L cells (Ryan and Heidrick, 1968). Otten, Johnson and Pastan (1971) studied fibroblasts in logarithmic growth phase and growth of virus transformed 3T3 cells in vitro; they found that intracellular levels of c-AMP were inversely proportional to DNA synthesis. The studies of Averner, Brock and Jost (1972) have indicated that c-AMP directly inhibits transcription and not replication of DNA. Under certain circumstances only does c-AMP appear to stimulate DNA synthesis and division; this occurs in cells that have already been programmed for growth by a promotional, as opposed to a mitogenic, effect (Rixon, Whitfield and MacManus, 1970).

It seems difficult to reconcile our observations that tumour CFE is stimulated in tissues which have been stressed by inflammatory reactions and β-adrenergic stimulation, which cause tissue c-AMP to increase, with the finding that c-AMP causes pleiotypic effects and initially inhibits growth in cells and tissues. This raises the possibility that “take” and survival of seeded cancer cells in tissues in the initial stages depend not so much on stimulation of mitogenesis as on other physiological changes which enhance adhesion and attachment of the cells to normal tissue (endothelial) surfaces and bring about a better physiological and cyto-architectural integration of the tumour cells with the host, to subserve their metabolic needs for the reprogramming of gene expression. This supposes that a preliminary modulation of tumour cell function occurs in which growth is temporarily held in abeyance—a change which is readily reversible and may be, perhaps wrongly, designated “differentiation” (Weiss, P., 1973) and which would be favoured by increases in tissue c-AMP. The mechanisms responsible for attachment of seeded cancer cells to endothelial and other tissue surfaces in vivo have received widespread attention; the roles of haemocoagulation and fibrinolysis in particular have been extensively investigated in this respect. However, a recent comprehensive quantitative study by Röttinger, Sedlacek and Suit (1975) has failed to show that anticoagulant therapy affects tumour growth in either normal or irradiated tissues, and treating mice with the defibrinating agent anecrod did not significantly affect clonogenic growth or spread of subcutaneously implanted tumour cells (Peters and Hewitt, 1974). Increases in the fibrinolytic activity and clotting of blood have been reported to occur after the injection of adrenaline and in stress (Biggs, MacFarlane and Pilling, 1947); evidence of a basic diurnal
rhythm of fibrinolytic activity has been obtained also (Fearnley, Balmforth and Fearnley, 1957). Under certain conditions, for obscure reasons, deposition of fibrin and fibrinolysis do appear to affect survival and growth of implanted tumour cells (Peters and Hewitt, 1974) but treatment with anticoagulants does not significantly reduce the effects of x-irradiation and various other stressors of stimulating tumour CFE in the lungs of rats (van den Brenk et al., 1973a). Recent studies have shown that early interactions between tumour and host, which lead to attachment and the provision of adhesion gradients for directional migration (haptotaxis; Carter, 1967), invasion and clonogenic growth of cancer cells in tissues depends on chemically mediated cell-to-cell signals which modulate interactions between surface proteins. Cyclic nucleotides appear to act in this way as chemical signals which govern contact inhibition and confluence of growth and so-called “differentiation”. Intracellular c-AMP concentrations have been found to increase with confluency and contact inhibition has been attributed to activation of membrane AC (Heidrick and Ryan, 1970, 1971). The adhesion of Ehrlich ascites tumour cells to a plastic surface was not affected by c-AMP; this finding was attributed to the presence of PD in the serum used since PD resistant dibutyllyr c-AMP, applied as a continuous signal, decreased adhesion (Weiss, L., 1973). More recently, evidence has been obtained that c-AMP, applied intermittently to cells in culture as pulses, provides a chemotactic signal whereby the cells communicate during aggregation (Shaffer, 1975; Gross, 1975), and that their exposure to a short pulse of c-AMP causes them in turn to secrete c-AMP. This cell–cell reaction induces physiological modulation of a cell population from a state of active multiplicative growth to one of “aggregation competence”. We postulate that the attachment of a seeded tumour cell to an endothelial surface may be an aggregation phenomenon which similarly requires the generation of a pulsatile c-AMP signal by the tumour bed (endothelium) for the tumour cell to become structurally and physiologically assimilated and nurtured by the sharing of a common micro-environment. Thus, Lettré (1952) showed that ATP caused enhanced flattening and adherence of cells to surfaces, even when the cells were in mitosis. Weiss (1961) has argued that such cellular expansion at an interface is a metabolically “active” rather than passive process. The higher net negative charge on transformed cells than on normal cells (Abercrombie, Heaysman and Karthausen, 1957) also would be expected to facilitate adhesion of the cancer cell to endothelium. Thus, it has been shown that when transformed cells are seeded in vitro on a monolayer of untransformed cells in the absence of a plasma coagulant, they do not form the heaped up colonies seen on glass, but spread as a layer over the normal cells (Vogt and Rubin, 1961). Interestingly enough, similar experiments by Stoker (1964) showed that the rate of cell division decreased during this form of spreading and migratory growth of transformed cells on normal cells—a finding which would be expected if this modulation of growth was mediated by a c-AMP signal. Consequently, the enhancement of “take” and replicative growth of tumour cells in vivo by stressors may depend on a biphasic mechanism, in which stimulation of c-AMP synthesis in normal tissue in the first instance causes adherence and aggregation competence of the tumour cells and secondarily induces the secretion by normal tissues of GSS which stimulates tumour cell mitogenesis and replication, i.e., a sequence of physiological changes which would appear to be in keeping with a “Yin and Yang” (Goldberg et al., 1972) or “see-saw” (Rudland et al., 1974) hypothesis, in which c-AMP and c-GMP have antithetical actions in the regulation of growth as well as in other bi-directionally controlled physiological processes.

The two key metabolic pathways which are involved in the cyclic nucleotide “see-saw” and lead to cell synthesis of the
two "antagonistic" messengers c-AMP and c-GMP are linked by inosine-5'-monophosphate (IMP)—the nucleotide which occupies a strategic position in purine metabolism; changes in the regulation of IMP synthesis by IMP-dehydrogenase which occur in states of regenerative and neoplastic growth have recently been linked with competitive routes for utilization of IMP in the "expression of degrees of malignancy" (Jackson, Weber and Morris, 1975). A GSS fraction has been prepared from brain which has a biphasic effect on myocytes in vitro, comprising induction of proliferative growth accompanied by differentiation (Gospodarowicz, 1975)—"antagonistic" actions in this respect which resemble the actions of the β-adrenergic agent ISOP in salivary tissues in vivo and in which cyclic nucleotide metabolism plays a key role. Beta-adrenergic agents were relatively less effective in stimulating tumour CFE in lung than inflammatory reactions. This may be due to the more prolonged action (chronicity) of the inflammation in causing cyclic nucleotide malfunction than that of injecting rats with a rapidly metabolized β-adrenergic drug. This is supported by the finding that treatment with the PD inhibitor, aminophylline, which increases and prolongs the effect of β-adrenergic agents on tissue c-AMP, also increased their effect (and that of other stimulants) on tumour CFE (Table III, Fig. 3).

Tumour CFE was stimulated by both α- and β-adrenergic blocking agents; the latter did not significantly alter the stimulating effects of β-adrenergic agonists on CFE. These findings do not necessarily conflict with the view that activation of receptor bound AC stimulates tumour growth, since β-blocking agents stimulate c-AMP activity in certain tissues (Allison, Denman and Barnes, 1971) and do not effectively block some β-adrenergic receptors (Gillis, Pearle and Hockman, 1974). In vitro, solubilized AC can be activated by fluoride (Schramm and Naim, 1970) and imidazole stimulates c-AMP-phosphodiesterase activity in subcellular fractions (Sutherland and Rall, 1958), but neither fluoride nor imidazole appears to be active in vivo (Robinson et al., 1971). This would explain their failure to alter tumour CFE in rats treated with β-adrenergic agonists.

The anti-adrenergic agents, guanethidine and bretylium decreased tumour CFE in rats; they also decreased the effects of adrenaline on CFE but did not significantly decrease stimulation of CFE by inflammatory reactions induced by cellulose sulphate or x-irradiation, which are mediated by the release of non-adrenergic agonists. Depression of tumour CFE by guanethidine and bretylium would appear to be primarily due to the widespread depletion and decreased release of adrenergic transmitter substances stored in the peripheral and ganglionic nerve terminals. This suggests that in states of stress, apart from the release of adrenergic hormones, autonomic nerve stimuli may be directly involved in modulating tissue functions which affect tumour growth.

Cyclic AMP participates in the early events of the immune reaction in vitro (Bösing-Schneider and Kolb, 1973). Principally, it prevents spleen lymphocytes differentiating into antibody producing cells. The time scale for the action of c-AMP on immune induction which precedes proliferation of immunocytes is at least 24 h (Bösing-Schneider, 1975). Since the majority of tumour cells which seed in the lungs succumb within the first 24 h, it seems unlikely that the increased c-AMP synthesis in states of stress stimulates tumour growth by suppressing the development of active immunity.

Since it is likely that topical and systemic stress influences the metastatic spread of spontaneous and induced tumours in mammals in the same way as it affects experimentally seeded cancer cells, it follows that psychosomatic factors may influence the natural history of cancer in man to a greater extent than is commonly acknowledged. Also, the possibility
arises that a variety of therapeutic agents can affect growth of metastases; in repeated doses, certain cytotoxic agents such as cyclophosphamide increased tumour CFE in rats (unpublished results). Other non-cytotoxic but pharmacologically active agents may do likewise but may be more specific in this respect in singling out those target tissues which are endowed with receptors to the particular agonist. For example, although glucagon did not stimulate tumour CFE (or c-AMP synthesis) in the lungs, the possibility arises that it may do so in the liver, where glucagon causes a c-AMP mediated hyperglycaemic effect. Conversely, a drug which causes stress can indirectly stimulate tumour growth in those tissues which react weakly or not at all to the agonist, but react to adrenaline (e.g. stimulation of CFE in the lungs by 5-hydroxytryptamine; Table VI).

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