laboratory concerned with the lymphocyte origin in lymphatic leukaemia induced in the C57BL and C_3H strains of mice indicate that radiation and virus induced leukaemia in these strains of mice were also of T lymphocyte origin, whereas all DMBA induced leukaemias so far tested were found to be derived from bone marrow lymphocytes. Our results differ from those obtained by Shevach *et al.*[16], who analysed 21 mouse leukaemias, including some chemical carcinogen induced cell lines, and found that none of them could be classified as B lymphocyte leukaemias.

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Co-carcinogenic Effect of Progesterone on 20-Methylcholanthrene Induced Cervical Carcinoma in Mice

THE secretion of mucosubstances in the genital tract seems to be related to the ovarian output. It is well known that ambivalent cells of the vaginal squamous epithelium keratinize under oestrogenic action and mucify when progesterone acts synergically with oestrogen. This last condition seems to be required, at pro-oestrus, a few hours before ovulation. Direct evidence of oestrogen secretion at early pro-oestrus[1,2] and of progestin surge at mid-pro-oestrus in mice[3] and in rats[4] has been demonstrated.

At pro-oestrus, however, mucosubstances appeared in the cells of the genital tract of control C57BL6 mice. In this experiment we have shown that these mucosubstances were histochemically different. Acid and carboxyl mucosubstances were secreted by vagina and exocervix, but the uterine columnar epithelium secreted neutral mucosubstances, while the endocervical mucosal epithelium did not produce any glucidic secretion. In the course of this study, mucosubstances were systematically looked for in the squamous epithelium and were shown by the following histochemical reactions: PAS, salivary amylase, alcian blue (*p*H 2.6 and 1).

Progesterone administered for 9 weeks by subcutaneous implantation of pellets with a dose of 15 mg/mouse/3 weeks, to 25 C57BL6 3-month-old mice induced a strong intracellular reaction of mucification along the vaginal mucosa to exocervix. In the endocervical squamous epithelium, acid and carboxyl mucosubstances appeared only in few foci of cells. As, however, no glucidic secretion was exhibited in the endocervical cells during the normal oestrous cycle, it can be concluded that parenteral administration of progesterone induced a mucin-producing metaplasia in the endocervical mucosa.

Local exposure of carcinogen, 20-methylcholanthrene (MC), in the cervical canal for 9 weeks, in 50 C57BL6 3-month-old mice induced one invasive carcinoma in the vagina–exocervix and five in the endocervix.

Parenteral administration of progesterone to 50 C57BL6 mice for 9 weeks at the dose mentioned above was associated with carcinogenic action and significantly increased the total number of invasive squamous carcinoma in vagina–exocervix and in endocervix, as shown in Table 1.

Table 1 Histological Types of Invasive Squamous Cell Carcinoma Appearing in the Vagina–Exocervix and Endocervix after Local Application of 20-Methylcholanthrene (MC) with and without Progesterone (P) after 9 Weeks Treatment

Invasive squamous cell carcinoma	Vagina–exocervix			Endocervix		
	MC	MC+ P	*P* value	MC	MC+ P	*P* value
Immature microcarcinoma	0	9	0.01	3	9	n.s.
Keratinized	0	1	n.s.	1	0	n.s.
Mucin-secreting	1	5	n.s.	1	21	0.01
Total number of invasive squamous cell carcinoma	1	15	0.01	5	30	0.01

Histochemical and histological examination of a hundred specimens under study showed that in our experimental conditions progesterone selectively influenced the maturation of the induced endocervical invasive carcinoma, gave a mucoepidermoid type ($P < 0.01$) and had no effect on the maturation of induced invasive carcinoma from the vagina and the exocervix which remained immature.

Our results would indicate that endocervix in the strain of mice used might be a target organ for the action of progesterone or for its metabolites.

The progestative hormone, by inducing a mucin-secreting metaplasia in the target organ, could make it sensitive to the action of the carcinogen, and would thus influence the histological type of the induced carcinoma. The role of progesterone on the carcinogenesis of the cervix has received very little attention[5,6]. Kaminetzky[7] noted that progesterone administration advanced cervical dysplasia induced by 20-methylcholanthrene and Glücksmann and Cherry[8] drew attention to the fact that progesterone treatment did not affect the total number of induced tumours but increased the incidence of adenocarcinoma secreting mucus. Our findings suggest the possibility that a prolonged and high dose of progesterone or progestins which are known to provoke a strong stimulation of human endocervical glands[9,10] could promote carcinogenic activity of the agent primarily responsible for cervical tumours in women and tend to favour selectively the development of the mucoepidermoid maturation of the cancerous cells.

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Cholera Enterotoxin: Failure of Anti-inflammatory Agents to Prevent Cyclic AMP Accumulation

THERE is considerable evidence that cholera diarrhoea is caused by accumulation of cyclic AMP in intestinal mucosa, stimulated by cholera enterotoxin[1–3]. Prostaglandin-E_1 (PGE_1) reproduces the effects of cholera enterotoxin on both cyclic AMP and secretion of fluid and electrolytes by intestinal mucosa[3] and it was suggested[4] that the toxin might first act by stimulating the release or synthesis of prostaglandin which then acts through cyclic AMP. Bennett[4] proposed that acidic anti-inflammatory drugs, as they are potent inhibitors of prostaglandin synthesis in several tissues[5–7], might antagonize the effect of cholera enterotoxin. Jacoby and Marshall reported that indomethacin, aspirin, and several other anti-inflammatory agents inhibited the cholera toxin-induced accumulation of fluid in ileal-ligated rats[8]. Because of its potential therapeutic significance, I have studied this proposed mechanism.

The cyclic AMP system is stimulated by cholera enterotoxin in virtually every tissue so far tested, including liver, adipose tissue, and blood platelets, in addition to intestinal mucosa[1,9–11]. Low concentrations (1–10 ng $ml.^{-1}$) of the purified toxin increased adenyl cyclase activity and cyclic AMP accumulation in human leucocytes[12,13], and this effect was similar in all respects to that on intestinal mucosa, including toxin concentration required, the characteristically delayed time-course, and specific inhibition by cholera toxoid and a canine antitoxin. The leucocyte seemed a suitable tissue for testing Bennett's suggestion *in vitro*, as PGE_1 also stimulates leucocyte cyclic AMP accumulation[13–15].

Leucocytes of three healthy human volunteers were prepared as described previously and suspended in a Tris-buffered salt solution containing 0.3% (w/v) human serum albumin[14]. Leucocytes (1×10^7 $ml.^{-1}$) and purified cholera enterotoxin 10 ng $ml.^{-1}$, a maximally effective concentration[12]) were incubated for 90 min at 37° C. Varying concentrations of indomethacin or aspirin were added to leucocytes 5 min before addition of cholera toxin. Incubation was terminated by centrifugation (2,000*g* for 1 min), after which the supernatant fluid was discarded and the cell button resuspended in ice-cold 5% trichloroacetic acid. Leucocyte cyclic AMP was then assayed by the competition-binding method of Gilman[16], modified as described previously[15].

Table 1 Leucocyte Cyclic AMP after Exposure to Anti-inflammatory Agents and Cholera Enterotoxin

	Cyclic AMP, picomol per 10^7 cells *		
	Exp. 1	Exp. 2	Exp. 3
No drug	4.4	7.2	5.6
Indomethacin 70 μg $ml.^{-1}$	5.0	10	7.4
Aspirin 700 μg $ml.^{-1}$	—	9.0	8.0
Cholera enterotoxin 10 ng $ml.^{-1}$	39	75	45
+ Indomethacin 70 μg $ml.^{-1}$	41	84	44
+ Indomethacin 7 μg $ml.^{-1}$	38	69	43
+ Indomethacin 0.7 μg $ml.^{-1}$	37	72	44
+ Aspirin 700 μg $ml.^{-1}$	—	76	39
+ Aspirin 70 μg $ml.^{-1}$	—	73	40
+ Aspirin 7 μg $ml.^{-1}$	—	67	43
+ Aspirin 0.7 μg $ml.^{-1}$	—	68	42
PGE_1 1×10^{-5} M	32	50	40

* Each value is the mean of duplicates differing by ±8%.

Neither aspirin nor indomethacin produced any detectable inhibition of the eight-fold increase in leucocyte cyclic AMP caused by cholera enterotoxin (Table 1). PGE_1 alone caused accumulation of cyclic AMP, as described previously[13–15]. The maximal concentrations of both anti-inflammatory agents were considerably higher than those which produced complete inhibition of prostaglandin synthesis in guinea-pig lung[5] and canine spleen[7]; 3–4 μg $ml.^{-1}$ for indomethacin and 30–40 μg $ml.^{-1}$ for aspirin.

The similarities between cholera enterotoxin's effects on the cyclic AMP content of gut mucosa and leucocytes suggest that the toxin's mechanism of action may be similar in the two tissues. If so, high concentrations of aspirin and indomethacin would not be expected to prevent accumulation of cyclic AMP in gut mucosa exposed to maximally effective doses of cholera enterotoxin. This possibility must be tested directly with maximally—and submaximally—effective doses of cholera toxin on the gut mucosa, and it must be shown in both leucocytes and mucosa that indomethacin and aspirin greatly reduce prostaglandin synthesis. The present results show that cholera toxin alone can increase cyclic AMP levels maximally in human leucocytes. They do not exclude the possibility that the gut behaves differently or that a dual mechanism exists. In addition to the direct action of toxin there might be an indirect effect involving prostaglandins, and this would become evident only in experiments with the submaximal doses of toxin. Nevertheless, increased synthesis of prostaglandin is unlikely to prove a universal mechanism for the actions of cholera enterotoxin, since the toxin (like cyclic AMP) stimulates lipolysis in adipocytes[9], cells in which PGE_1 inhibits both cyclic AMP accumulation and lipolysis[17,18].

These results do not preclude a beneficial therapeutic effect of acidic anti-inflammatory agents in cholera. The inhibition of toxin-induced intestinal fluid accumulation by these agents *in vivo*[8] deserves further investigation, even if it belongs to a class of drug effects in which cyclic AMP plays no role.

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