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Subcutaneous Growth of Human Tumours in Mice

CASTRO¹ reported the successful growth of human tumours in immune suppressed mice after the tumour tissue had been implanted beneath the left kidney capsule. In similar work we have used the subcutaneous route because we found this to be the most successful in our extensive investigations into tumour growth in ALS treated mice.

Female CBA mice were thymectomized at 4 weeks, whole body irradiated (900 R), 2 weeks later and reconstituted within 3–4 h of irradiation, using 1×10^7 syngeneic bone marrow cells, given i.v.

Table 1 Subcutaneous Tumour Growth

Tumour type	Percentage survival of tumours				
	1 wk	2 wk	3 wk	4 wk	5 wk
HeLa	100	100	100	53	21
Colon	100	100	100	80	
Stomach	100	100	50	38	38
Hep 2	75	75	50	50	12
Rectum	100	75	50	50	
Caecum	100	100	50	50	25
Breast adenocarcinoma	0	0	0	0	0
Secondary tumour, the primary being in the breast	70	70	70	70	
Lymph node from melanoma	100	100			

The tumours were obtained at operation and transported to the laboratory in Eagles minimal essential medium, containing 2% calf serum, 200 units of penicillin, and 100 mg streptomycin. Tumour pieces of about 2 mm were implanted subcutaneously in a ventro/lateral site over the right hip joint. In our ALS experiments we found by experience that this was the most suitable site for implantation, in view of the large tumour growths obtained, often in excess of 2–3 cm. In our present investigations we are able to report colonic tumour growths of the order of 12 mm within eleven days of implantation. This was assessed by direct palpation. Tumour types investigated are given in Table 1.

Our results clearly show that the subcutaneous route pro-

duces good tumour growth which is easy to assess by direct palpation and therefore affords a means of judging the effectiveness of treatment without having to kill the mice. We feel this method will be useful for further *in vivo* investigations of human tumours.

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Inhibition of Pupariation in *Sarcophaga bullata*

THE classical test used as the bioassay for the insect moulting hormone, ecdysone, is based on the failure of the posterior part of ligatured larvae of *Calliphora erythrocephala* (or other fly species) to tan unless an adequate dose of ecdysone is administered. It is crucial for the success of the test to select larvae at the right stage of development, and as closely as possible to the critical time for pupariation¹. Larvae ligatured before the critical stage of natural ecdysone release may eventually pupariate in the front part where the hormone originates, but will never do so in the hind part. Post-critically ligatured larvae have had sufficient ecdysone released into the haemolymph previous to ligature to induce tanning in the hind parts. It is evident that at this time the anterior parts must also be post-critical. In larvae ligatured at this stage the anterior parts usually contract to the puparium and tan whereas the posterior parts merely tan without change in shape. The occurrence of tanning in the posterior part alone is therefore a paradox. Fraenkel² along with the description of the "*Calliphora* assay" also reported that ligatured larvae occasionally tanned only in the posterior region. Several investigators have been subsequently surprised by this phenomenon of "anterior inhibition", but there has been no agreement as to its cause.

Kaplanis *et al.*³ observed anterior inhibition in the larvae of *Musca domestica*. Langley⁴ attributed anterior inhibition in the larvae of *Glossina morsitans* to an inhibitory influence of the central nervous system which in a ligatured larva is confined to the anterior part. In contrast, Price⁵ hypothesized the presence of an inhibitory factor of the phenol oxidase inhibitor type, to account for a temporary inhibition of pupariation in the anterior parts of *C. erythrocephala* larvae ligatured between segments 8 and 9. Fraenkel and Zdarek¹ reported that in different species of flies the percentage of anterior inhibition is different. In the study reported here, with *Sarcophaga bullata*, it varied, according to the condition of the experiment, between 0 and 80%. Chang⁶ observed a 96.9% anterior inhibition in *Lespesia archippivora* that had been in his view ligatured "pre-critically", and suggested that, contrary to common notions, ecdysone was produced in the posterior parts only. The injection of ecdysone into such anterior parts did not, however, induce tanning. In the absence of evidence about the pre-critical nature of his larvae we suggest that they were indeed ligatured post-critically and showed the same effect of anterior inhibition observed in the other species.

Our studies now confirm Fraenkel's original view² that the inhibition of pupariation in post-critically ligatured larvae was