leukaemia a similar bi-modal seasonal distribution was observed.[1] The existence of two peaks was noted whether the time of clinical onset was studied, or the moment of diagnosis, or that of death. The same two peaks, one in winter and another in spring, were present when the total morbidity—i.e., the number of cases under observation at any given time—was plotted. During the discussion of our paper at the Lisbon Haematological Congress' two Continental haematologists stated that their findings were of a similar character.

The bi-modal seasonal variation of certain types of leukaemia may be fruitfully compared with that of other neoplastic diseases and diseases of viral aetiology.—I am, etc.,

A. SWAN.

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London S.W.12.

REFERENCE

1 Swan, A., Petrelli, M., and Surtees, S. J., in *Proceedings of the 9th Congress of the European Society of Haematology, Lisbon, 1963*. Karger, Basle and New York. In press.

A Dangerous Magpie

SIR,—A patient of mine, hearing her 5-month-old baby screaming in her pram in the garden, rushed out to find an adult magpie perched with a strong grip on the child's right forearm and pecking at the child's eyes. So determined was the attack that the bird was only driven off with difficulty. There were minor abrasions on the eyelids and scratches on the forearm, none of which was serious.

In 30 years' rural practice this is the first time I have ever heard of such an attack. I thought the incident might be worthy of record.—I am, etc.,

Winster, Derbyshire. A. D. STOKER.

Sodium Fluoride and Cell Growth

SIR,—As there has recently been controversy in the Oxford area regarding the addition of fluoride to the city water supply we felt it appropriate to study the effects of sodium fluoride in concentrations comparable to that proposed for the water supply upon cells in tissue culture. Two lines of mammalian cells were used: (1) HeLa S-3_{oxf}, derived from a patient with carcinoma of the cervix and acquired from Dr. Charles Ford, M.R.C. Radiobiological Research Unit, Harwell, in 1962. This line was grown in medium 199 (Glaxo) supplemented with 10% fresh human AB serum. (2) A clone of strain L mouse fibroblasts obtained from Dr. John Paul (Glasgow) and sub-cloned in this laboratory by Dr. A. H. W. Nias. These cells were grown in medium 199 supplemented with 2% pooled calf serum (Oxoid) and 0.5% Bacto-Peptone (Difco) for growth curve experiments or with 10% pooled calf serum for plating experiments. Both lines of cells were grown in sealed 12-oz. (340-ml.) bottles (medical flats), 20 ml. medium per bottle, 5×10^5 to 1×10^6 cells per bottle initial inoculum, or in sealed 6-oz. (170-ml.) bottles (medical flats), 10 ml. medium and $1–5 \times 10^5$ cells initial inoculum per bottle. Growth curves were determined by counting the number of cells per bottle after various periods of incubation at 37° C.; the medium was poured off and the cells trypsinized from the glass surface, diluted immediately 1:50 with ice-cold normal saline solution and counted in a Coulter Model D electronic cell counter which had previously been calibrated against haemacytometer counts under phase microscopy. In all experiments duplicate bottles were counted for each experimental point.

In every experiment in which sodium fluoride in concentration of 0.1 mg./l. (1/10 p.p.m.) or more was added to the medium the growth of both these cell lines was appreciably depressed (see Table). Additional sodium chloride in concentration equal to the highest dose of sodium fluoride used did not depress the growth rate.

In an attempt to determine whether the depression in growth observed in these cell lines was due to direct lethal effects, the cells were grown in clonal culture after the method of Puck *et al.*[1] In these experiments known numbers of single cells were inoculated into Falcon polystyrene 5 cm. T.C. petri dishes in appropriate medium and incubated in a humidified 5% CO–air atmosphere for 14 days. The dishes were then fixed and stained with Leishman's stain and the number of macroscopically visible clones of cells counted (clones of more than 30 cells scored as reproductively intact and viable). In these experiments *no* significant reduction of cell reproductive capacity was detected at concentrations of sodium fluoride up to 10 mg./l. (10 p.p.m.); however, at 100 mg./l. (100 p.p.m.) there was total disappearance of viable cells from the plates.

Per cent. of Control Growth in Seven Days

Cell Type	Concentration of Sodium Fluoride in Medium		
	0·1 mg./l. (1/10 p.p.m.)	1·0 mg./l. (1 p.p.m.)	10·0 mg./l. (10 p.p.m.)
Human Carcinoma (HeLa S-3_{oxf})			
Experiment 1	—	72·5	69·8
2	82·5	89·8	73·0
3	92·5	90·2	70·9
4	85·5	85·9	75·5
Average	86·8	84·6	72·3
Mouse Fibroblast (L, clone 1^2/oxf)			
Experiment 1	—	78·4	72·6
2	68·5	77·0	53·0
3	95·0	81·1	65·7
4	92·1	94·3	65·6
Average ..	85·2	82·7	64·2

The growth of two types of mammalian cells *in vitro* has thus been shown to be inhibited by extremely minute quantities of sodium fluoride in the growth medium—quantities equivalent to those recommended for use in drinking-water. As significant decrease in cell reproductive capacity does not occur until very much higher fluoride concentrations are reached, this reduction of growth is probably due to a decreased rate of cell division, not to direct and immediate cell-killing.

R. J. B. is Helen Hay Whitney Fellow in Radiobiology at Oxford University.

We should like to acknowledge the continuing support of the Damon Runyan Memorial Fund for the work of this laboratory and the enlightened guidance of Dr. Frank Ellis, Director of the Department of Radiotherapy.

—We are, etc.,

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Radiotherapy Department, and
The Pharmacy,
Churchill Hospital, Oxford.

REFERENCE

1 Puck, T. T., Marcus, P. I., and Cieciura, S. J., *J. exp. Med.*, 1956, **103**, 653.

Blood Supply of Long Bones

SIR,—In an article in the *Journal of Bone and Joint Surgery* for May, 1963, Professor J. Trueta examines the question of a possible periosteal arterial supply to the cortex of long bones.

Many of your readers will no doubt be aware that several recent investigators[1–4] have concluded that, in normal circumstances, a periosteal arterial supply to the compact bone of the shaft is negligible in amount, and that the blood flows from the medulla out through the cortex into the periosteum. In other words there is a single circulatory system in compact bone and the direction of the blood flow is centrifugal. Professor Trueta writes, however, that the outer third is supplied by periosteal arteries and the inner two-thirds by medullary arteries. This older view implies that there are two separate circulatory systems in bone cortex each possessing its own arterial supply, capillary field, and venous drainage. These peculiarities have never been demonstrated anatomically. As evidence for his statement, Professor Trueta briefly describes his experiments, of the type so beautifully executed by Professor Marneffe,[5] wherein the outer compactum survived destruction of the medullary supply, or died following suppression of the periosteal blood flow. As has been fully explained elsewhere,[6] these pathological findings are not in conflict with the known facts relating to the normal vascular anatomy of bone. In brief, these show that a unified cortical capillary field is regularly interposed between periosteal capillaries and venules externally and medullary arteries internally. Hence an anatomical basis is to hand whereby, for example, suppression of the medullary supply allows blood from the periosteum to pass into the cortex and so exercise the function of a collateral blood route to bone.