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 Congress¹ 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.,

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REFERENCE

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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 haemocytometer 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% CO₂-air atmosphere for

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

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.

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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REFERENCE

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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 investigators¹⁻⁴ 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.

Those who support the concept of centrifugal flow in the cortex point to the great rarity of arteries in the outer third, which have never been visually demonstrated even by writers who have diligently sought them out,⁷⁻⁹ despite the apparent ease with which they could be filled, if they existed. Professor Trueta in his article has claimed to demonstrate them by electron microscopy. His photographs, which presumably are his best, are printed as negatives, contrary to accepted practice, and are therefore difficult to assess, especially without benefit of technical details, such as mode of fixation and precise site of origin of the ultra-thin sections. In the knowledge that these are prone to artifact formation even in the best of hands, and open to misinterpretation, the interested reader will come to his own opinion as to their evidential value.

Another technique used by Professor Trueta to show periosteal arteries in bone cortex, the tetracycline injection method and ultra-violet microscopy, gives, if anything, additional proof of the regular capillary nature of the vessels in compact bone, a fact in support of the centrifugal flow concept and anyway far better and more easily demonstrated by Indian ink perfusion. It is surprising that Professor Trueta should refrain from demonstrating periosteal arterial channels regularly passing into the outer cortex of normal bones, using the perfusion technique of which he is rightly an acknowledged master. This should not be so difficult; even a small capillary is 5 μ in diameter and can be seen easily by medium power light microscopy. Until arterial channels are demonstrated to be a regular feature of the outer compactum, the concept of centrifugal flow in the cortex of long bones, as originally enunciated in 1957,¹ may be said to reflect the facts of cortical circulation better than the older view supported by Professor Trueta.—I am, etc.,

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Gall-bladder Dyspepsia

SIR,—I hope you will forgive me if I disagree with you when you say in your leading article (July 20, p. 128) "a further diagnostic bubble is burst" on the strength of Dr. W. H. Price's article (July 20, p. 138) in the same number. He seems to rely on x-rays for the diagnosis,

and both he and Dr. A. H. Douthwaite (August 3, p. 318), following Moynihan, seem to stress gall-stones as the cause of the dyspepsia. Herein, I think, lies the fallacy; it is the disease of the gall-bladder, not the stones, which causes the dyspepsia, and x-rays only play a small part in the diagnosis. Dr. Douthwaite rightly lays stress on the symptoms.

John Ryle,¹ a very reliable and astute observer, divided the dyspepsias into five groups, according to causes, (1) habit, (2) nervous, (3) toxic and infective, (4) irritative (ulcer, infections of the gall-bladder and appendix), (5) mechanical (pyloric stenosis, cancer, etc.). Of 267 cases, unselected except that they had been ill enough to go to hospital or a consultant, he found that 52% fell into groups 4 and 5. My friend Arthur Hurst inspired me with a special interest in these groups where surgery and medicine overlap so much in treatment, and gave me much help and advice.

The diagnosis of chronic cholecystitis depends on the symptoms; distension after meals, goose skin or shivering, and referred pain and tenderness in areas, front and back, supplied by seventh, eighth, and ninth segments of the spinal cord. The symptoms may not be typical, and may indeed resemble those of duodenal ulcer. The gall-bladder is tender, and there may be guarding of the right rectus. It is only when the disease is so far advanced that the gall-bladder fails to concentrate dye, or contains stones, that a cholecystogram shows abnormality. Gall-stones, I believe, only cause symptoms when they cause obstruction, or when there is, as so often, infection present. Indeed, Hurst² thought that Moynihan's "inaugural symptoms of gall-stones" were the symptoms of cholecystitis, and he thought that if x-rays showed want of concentration in the gall-bladder the case was beyond medical treatment.

If medical treatment has failed, or is contraindicated, then operation is justified, and operation gives more satisfactory results. If there is no such entity as gall-bladder dyspepsia, it is difficult to explain the success of cholecystectomy in ending the dyspepsia in cases of cholecystitis.—I am, etc.,

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Different Patterns of Human Porphyria

SIR,—As a worker who for many years has been engaged in the study of porphyria in man, as well as cattle and pigs, I feel that a few additional remarks to the important contribution of Professor Jan Waldenström and Dr. Brigitta Haeger-Aronsen (August 3, p. 272) are needed. Firstly it is important to keep in mind that "porphyrias" is not a precisely defined term. Goldberg and Rimington¹

write that porphyrias are metabolic disorders, hereditary in origin, but nevertheless they also use the term to designate acquired conditions.² It is therefore not logical to define porphyrias as inborn errors of metabolism; if the nomenclature now in common use is accepted, both hereditary and non-hereditary porphyrias must be accepted. Secondly, Professor Waldenström and Dr. Haeger-Aronsen forgot to mention two forms of hereditary porphyria in man, idiopathic hereditary coproporphyria³ and infantile haemolytic anaemia accompanied by excretion of porphyrin precursors porphobilinogen (P.B.G.) and δ -aminolaevulinic acid (A.L.A.).⁴ These two hereditary metabolic diseases are especially interesting because the former is associated with an abnormal amino-acid excretion pattern and the latter forms a bridge between hereditary haemoglobinopathies and porphyrias. Recently I have pointed out⁵ that it may be more correct not to distinguish between porphyrias, haemoglobinopathies, and inherited anomalies of bile pigment metabolism and look on these inborn errors of metabolism—enzyme disorders, molecular diseases—as errors of pyrrole metabolism and not as three distinct groups. It is, however, to be remembered that our knowledge of these conditions is at present very limited. Therefore it is to be recommended that the porphyrin metabolism should be studied also in haemoglobinopathies and hereditary anomalies of bile pigment metabolism; in general, biochemical studies of as broad a nature as possible, together with genetical studies, should be performed in these conditions. In my opinion the nomenclature of the porphyrias is to-day very contradictory, with every investigator presenting his own variation. We have to admit that our knowledge is too limited to accept a rigid subdivision of the porphyrias, and various authors are very far from agreement. Personally, I agree with the interpretation of Professor Waldenström on the whole, but I want to point out that conditions are a bit more complicated than is expressed in the article of Professor Waldenström and Dr. Haeger-Aronsen. Thus I have seen two paralytic cases of acute intermittent porphyria where no P.B.G. could be demonstrated in the urine by ion-exchange chromatography, and I feel it is important to point out that there is no correlation between the excreted quantities of P.B.G., A.L.A., and porphyrin and the clinical symptoms in acute intermittent porphyria, a fact first pointed out by Ackner *et al.*⁶ Professor Waldenström and Dr. Haeger-Aronsen mention in their discussion that acute intermittent porphyria may be several genetically closely related but different molecular diseases due to various mutations of the same gene. In a study of the relatives of a series of cases of acute porphyria by means of ion-exchange chromatography of P.B.G. and A.L.A. I found several facts pointing in this direction. This material is to be presented in detail together with a thorough descrip-