attributed to myocardial inflammation (Wolff and Grundfeld, 1963). For this reason it does not seem justifiable to divide the cardiac manifestations due to viral infection into pericarditis and myocarditis; it would be more reasonable to speak of myopericarditis and myocarditis (Smith, 1966).

Among the clinical findings in the present cases leucocytosis and raised A.S.T. and S.G.O.T. should also be mentioned. The occurrence of leucocytosis in seven patients corresponds to the observations made in previous series of viral pericarditis. The A.S.T. was elevated in three out of 16 patients investigated. The same phenomenon has previously been noted in certain cases of pericarditis due to Coxsackie B5 virus (Gillett, 1959; Hedlund *et al.*, 1962; Pollen, 1963). The raised S.G.O.T. values in two patients seem to suggest severe myocarditis (Smith, 1966) or simultaneous anicteric hepatitis (Sun and Smith, 1966).

The pathogenesis of viral myopericarditis is not yet fully understood. Whether the heart muscle is affected directly or by some sensitivity or immunity reaction is still far from clear (Sanders, 1963). It has been suggested that the cardiac manifestations in viral disease may include endocarditis, which may lead to valvular lesions (Burch and DePasquale, 1964; Smith, 1966). The part played by viral myopericarditis as the primary cause of aetiologically obscure persistent cardiomyopathies has not yet been clarified. Long-term follow-up studies might yield valuable information.

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Preliminary Communications

Bone Marrow Colony-stimulating Activity of Sera in Infectious Mononucleosis

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Summary: From 44 to 100% of sera from patients with infectious mononucleosis exhibited the capacity to stimulate colony formation in vitro by mouse bone marrow cells. The proportion of sera with colony-stimulating activity was highest in patients with a short fever period and developing low Paul–Bunnell titres. Patients with a more severe course of the disease generally displayed no, or only weak, colony-stimulating activity in their sera, and also had higher Paul–Bunnell titres. The level of serum colony-stimulating activity tended to fall in the convalescent stages of the disease.

When suitably stimulated certain mouse bone marrow cells can proliferate in semisolid agar cultures and form colonies of granulocytic and mononuclear cells (Bradley and Metcalf, 1966; Metcalf *et al.*, 1967). Mouse serum has been shown to contain a factor (the colony-stimulating factor) which can stimulate the formation of such colonies (Robinson *et al.*, 1967). This factor is a relatively heat stable, non-dialysable substance migrating electrophoretically in the post-albumin region and having a sedimentation constant of about 4–7S (Stanley *et al.*, 1968). Levels of colony-stimulating factor in the serum are raised in mice with spontaneous or viral-induced leukaemia (Robinson *et al.*, 1967; Metcalf and Foster, 1967a) and are temporarily raised after infection with at least one virus—the lactate dehydrogenase-elevating virus of Riley (Foster *et al.*, 1968a).

A recent survey of human sera (Foster *et al.*, 1968b) has shown that normal sera do not possess detectable colony-stimulating activity for mouse bone marrow cells but that colony-stimulating activity is detectable in 15–70% of sera from patients with leukaemia and allied diseases, patients with proliferative disorders of leucopoiesis, and patients with acute non-bacterial infections.

In the course of the latter survey it was found that some sera from patients with infectious mononucleosis would stimulate colony formation. In the present study a detailed analysis has been made of serum colony-stimulating activity in such patients in relation to the clinical course of the disease and the occurrence of other abnormal findings in these patients.

Patients and Sera

The 38 patients with mononucleosis were young Swedish adults whose acute and convalescent sera were collected in 1966 and forwarded for diagnostic examination to the Department of Virology, National Bacteriological Laboratory, Stockholm. Either single (22 cases) or paired sera (16 cases) were taken from patients who fulfilled two criteria: (*a*) a positive Paul–Bunnell titre after absorption with guinea-pig kidney according to Davidsohn *et al.* (1951), and (*b*) a clinical course typical of mononucleosis with atypical mononuclear cells in the peripheral blood. The sera were kept for variable times at ambient temperature during shipment to Stockholm, and were kept at −20° C. thereafter. Thirty-nine of the 54 sera were subjected to heat inactivation at 56° C. for 30 minutes before testing. Previous experiments have shown that serum retains its colony-stimulating activity after freeze-thawing and that the active factor is stable when heated at 60° C. for 30 minutes. Normal human sera do not possess detectable colony-stimulating activity (Foster *et al.*, 1968b).

Bone Marrow Culture Technique

The bone marrow culture technique and the culture media have been described in detail elsewhere (Metcalf and Foster, 1967a). Bone marrow plugs were collected from a single femur shaft of three DBA/1 mice 2 to 3 months old and single cell suspensions were prepared by pipetting the plugs up and down in 5 ml. of bone marrow collecting fluid (40 ml. of double-strength modified Eagle's medium containing 20% foetal calf serum, 10 ml. of trypticase soy broth, and 50 ml. of water).

Usual cell counts were 4–6 × 10^6 nucleated cells per ml. Double-strength modified Eagle's medium, containing 20% foetal calf serum and 20% trypticase soy broth, was mixed with an equal volume of 0.6% agar in water (previously boiled for two minutes and held at 40° C.). The mixture was held at 37° C. and sufficient bone marrow cells were added to give a final concentration of 50,000 nucleated cells per ml. in the agar-culture medium. Sera to be tested were pipetted into 35-mm. plastic Petri dishes (Falcon Plastics, Los Angeles) in doses of 0.2, 0.1, and 0.05 ml. Into each dish was pipetted 1 ml. of the bone marrow suspension in agar medium. The serum was mixed thoroughly with the medium and the plates were allowed to gel at 20° C. for 20 minutes. Culture plates were then incubated without media change for 10 days in humidified incubators at 37° C. with a continuous flow of 5% CO_2 in air.

After incubation the plates were examined with a dissecting microscope and colony counts performed at ×30 magnifications. The criteria for scoring colonies have been described elsewhere (Metcalf and Foster, 1967a). Sera which stimulated the development of more than 5 colonies per plate at the 0.02-ml. dose level were scored as positive. In the results the number of colonies stimulated by a serum refers to the mean number developing in plates containing 0.2 ml. of serum. In each test run on mononucleosis sera, mouse sera of known colony-stimulating activity were included as controls.

Colony Morphology

Of the 54 mononucleosis sera tested, 31 (57%) showed significant colony-stimulating activity. All colonies stimulated were the typical loose aggregates of cells seen after colony stimulation by leukaemic mouse or human sera, and at 10 days after incubation each colony contained from 50 to 500 cells. Linear dose response relationships were observed between serum dose and the number and size of colonies developing. Each colony appeared to arise from a single cell and during the first four days of colony formation the colonies contained mainly granulocytic cells—myelocytes, metamyelocytes, and polymorphs—with a few phagocytic cells containing a large round, slightly eccentric nucleus. After four days there was a progressive disappearance of granulocytic cells from developing colonies and an overgrowth of the phagocytic mononuclear cells, such that by 10 days most colonies contained pure populations of mononuclear cells. These changes in cell populations in developing colonies were identical with those previously observed after colony stimulation by leukaemic mouse sera and active sera from humans with leukaemia or acute viral infections. No unusual cytoplasmic inclusions were noted in any colony cells, and no unusual cytoplasmic basophilia was noted in colony mononuclear cells.

Relation of Serum Activity to Serology and Clinical Course

When serum colony-stimulating activity was compared with the Paul-Bunnell titre a rough inverse correlation was observed (Table I). Thus 9/9 (100% sera with Paul–Bunnell titres of 1:80 or less showed colony-stimulating activity, whereas with sera having Paul–Bunnell titres of 1:640 or above, only 8/18 (44%) showed activity. The mean number of colonies stimulated by active sera in each group was similar. It was noted that all active sera with Paul–Bunnell titres of 1:640 or above had been taken from patients in the first two weeks of their illness. No sera with a high Paul–Bunnell titre taken later than two weeks after the onset of disease had any colony-stimulating activity.

The few sera tested from patients in the first few days of their illness showed weak or no colony-stimulating activity (Table II). Sera taken between 4 and 37 days after the onset of illness showed a fall with time in the percentage showing colony-stimulating activity.

TABLE I.—*Correlation of Serum Colony-stimulating Activity with Paul-Bunnell Titres and Fever Duration*

Paul–Bunnell Titre*	No. of Sera	Percentage of Patients with Fever ≥7 Days	Percentage of Sera with Colony-stimulating Activity
≤1 : 80	9	23	100
1 : 160	11	36	55
1 : 320	16	64	50
≥1 : 640	18	69	44

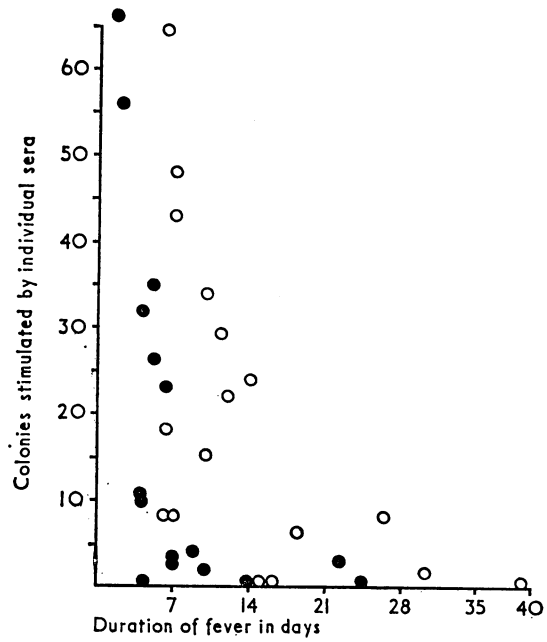
* Titres before absorption with guinea-pig kidney. All sera had specific titres after absorption with guinea-pig kidney.

TABLE II.—*Serum Colony-stimulating Activity Throughout Course of Mononucleosis*

Day After Onset of Illness	No. of Sera Tested	Percentage of Sera with Activity	Mean No. of Colonies Stimulated by Active Sera*
1–3	3	33	18
4–7	14	79	27
8–14	17	59	30
15–21	11	45	21
22–28	4	50	20
29–37	5	40	19

* Mean number of colonies in dishes containing 0·2 ml. of serum.

The clinical histories of the patients in the present survey were analysed to determine whether a correlation existed between the severity of the disease and serum colony-stimulating activity. The duration of fever was taken as a measure of the severity of the disease (Pejme, 1964). Table I shows that patients with a short duration of fever tended to have lower Paul–Bunnell titres and a higher incidence of sera with colony-stimulating activity. To analyse this further, the mean number of colonies stimulated by individual sera was plotted against duration of fever in that patient (see Chart). It can be seen that sera from patients with a short fever



Colony-stimulating activity in serum related to the duration of fever in patients with infectious mononucleosis. O Single sera. ● The more active serum from paired sera.

period tended to have the highest colony-stimulating activity, while no, or only weak, activity was found in sera from patients with a long fever course (>14 days). Though most sera in this analysis were collected in the acute stages of the disease, there was considerable variation in the actual day of illness on

which sera were collected. In retrospect, this analysis may have been more satisfactory if all sera had been collected at a uniform timepoint in the course of the disease.

No correlations were observed between serum colony-stimulating activity and the following clinical or laboratory data: age and sex of patient, lymph node or spleen enlargement, sedimentation rate, haemoglobin level, total white cell level, granulocyte or mononuclear cell levels, or the type of therapy given the patient (25 patients had a course of penicillin, 4 sulphonamides, 1 tetracycline, 1 chloramphenicol, 1 phenylbutazone, 1 cortisone, and 2 acetylic acid ; 10 were untreated).

In 16 patients acute and convalescent paired sera were available for study (Table III). These appeared to fall into two distinct categories. With eight patients, the acute sera showed activity which persisted in the convalescent sera in five and disappeared in three. In seven cases no colony-stimulating activity was detectable in either acute or convalescent sera. In only one instance was an acute serum inactive and the convalescent serum active, and in this case the acute serum was taken on day 2 of illness, possibly before colony-stimulating activity had a reasonable chance to develop. Of these 16 patients, the nine developing colony-stimulating activity in the serum had a short disease course, while of the seven failing to develop serum activity five had a prolonged disease course and two a short disease course.

TABLE III.—*Colony-stimulating Activity of Paired Acute and Convalescent Sera*

Group	Patient No.	Colony-stimulating Activity of*	
		Acute Serum	Convalescent Serum
Reactors	1	1	56
	2	6	32
	3	5	26
	4	23	16
	5	35	6
	6	67	9
	7	10	0
	8	11	0
	9	5	0
Non-reactors	10	0	0
	11	3	3
	12	0	4
	13	0	0
	14	2	0
	15	0	0
	16	3	1

* Mean number of colonies stimulated by 0·2 ml. of serum.

DISCUSSION

The colony-stimulating factor in mouse serum is tentatively regarded as a normal humoral factor regulating leucopoiesis (Robinson *et al.*, 1967 ; Foster *et al.*, 1968a). Though colony-stimulating activity is not detectable in normal human sera, levels of this factor are raised in leukaemia, in proliferative disorders of leucopoiesis, and in certain infections (Foster *et al.*, 1968b).

The present study has shown that from 44 to 100% of sera taken at random from patients with infectious mononucleosis exhibited a significant capacity to stimulate colony formation in vitro by bone marrow cells. In this respect mononucleosis sera exhibited activity similar to that of sera from patients with acute viral infections and acute leukaemia. In previous studies it has been shown that human mononucleosis sera cross-react with leukaemic mouse sera. Bone marrow colonies initiated by either type of sera have their growth supported when transferred to plates containing the other type of sera (Metcalf and Foster, 1967b). It has also been reported that bone marrow cells from patients with mononucleosis share with bone marrow cells from leukaemic patients the capacity to undergo lymphoblastoid transformation when cultured in vitro (Benyesh-Melnick *et al.*, 1968), but it is uncertain what relation this phenomenon has to the present findings.

It has earlier been shown that patients with clinical mononucleosis without a significant Paul–Bunnell titre have a shorter duration of fever than patients with high Paul–Bunnell titres (Pejme, 1964). This was confirmed in the present study. The duration of fever and the Paul–Bunnell titre can therefore be taken as measures of severity of the disease. The colony-stimulating activity of mononucleosis serum was inversely related to the severity of the disease, and the highest activity was found in patients with the shortest disease course. Of the 12 patients failing to develop serum activity, all except two had a prolonged clinical course. No obvious differences other than duration of fever were noted in the patients failing to develop serum activity.

It was not possible in the present survey to define the maximum duration of persistence of raised colony-stimulating activity in the serum of patients with mononucleosis. Three sera with low stimulating activity had no activity after 14 to 18 days, while no endpoint was established for highly active sera.

The development of colony-stimulating activity in the serum probably represents a response on the part of the patient to the infectious process. It may be that raised levels of colony-stimulating factor in the serum increase white cell proliferative activity in the bone marrow, which may be of benefit in limiting the duration of the disease. However, patients responding to the disease by developing high serum levels of colony-stimulating factor may respond well to the disease in other, unrelated, ways. Further studies are required to assess the possible value of high serum levels of colony-stimulating factor in limiting the duration of this and other infectious processes.

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