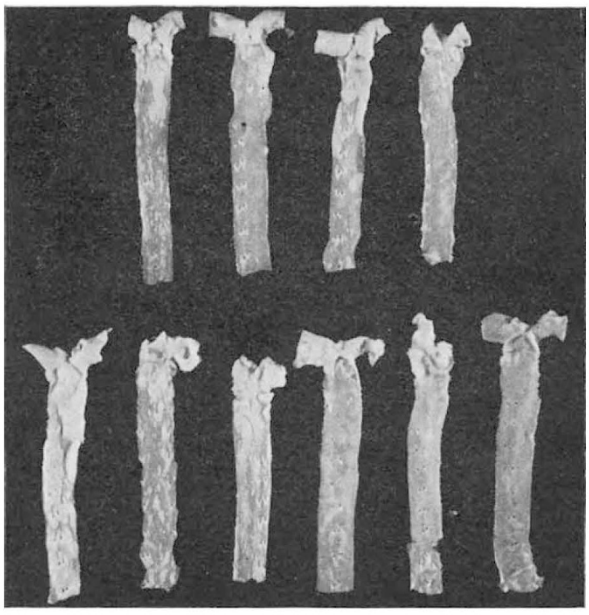

Fig. 2. Slit aortae of ten New Zealand white male rabbits which were fed a diet of 1 per cent cholesterol for 12 weeks and then transferred to a diet free of cholesterol. The six lower aortae are those of rabbits which then received a dietary supplement of 5 mg of cortisone acetate daily.

present was therefore examined. A group of ten rabbits was fed a diet of 1 per cent cholesterol for 12 weeks, sufficient for production of extensive plaques (Fig. 1). The rabbits were divided into two groups and transferred to a cholesterol free diet consisting of 100 g of plain pellets/day. The daily diet was supplemented with 5 mg of cortisone acetate for one group, and the other group was maintained as controls. After a further 12 weeks the plasma lipid concentrations had fallen to almost normal in the control group, but remained slightly larger in the animals fed cortisone (Table 2). Fig. 2 shows that there was no significant regression of plaques during the following 12 weeks in rabbits on a cholesterol free diet. Cortisone treatment also failed to induce any significant regression of plaques as compared with animals on the control diet.

These experiments indicate that the inhibitory action of cortisone in experimental atherosclerosis is not directly related to the accompanying plasma lipid changes and can be partially duplicated by a second anti-inflammatory agent (phenylbutazone) with no significant changes in the serum lipid pattern. These findings, together with the failure of cortisone to influence regression of plaques already deposited, support the idea[6] that the hormone acts in the early stages of plaque formation through its anti-inflammatory properties rather than through its lipaemic effects.

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J. MARTYN BAILEY
JEAN BUTLER

Department of Biochemistry,
George Washington University,
School of Medicine,
Washington, D.C.

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Culture of Human Leukaemia Cells

THIS communication describes the culture of four additional cell lines derived from the buffy coats of patients with leukaemia. Iwakata and Grace[1] provided key information for culturing leukaemia cells and reported the establishment of a cell line, R.P.M.I. No. 6410. Fifteen cell lines were subsequently derived from the buffy coats of four patients with acute and chronic myelocytic leukaemia and reported[2].

We have lately been able to establish cell lines from four additional patients with myelocytic leukaemia. Table 1 summarizes data concerning the human leukaemia cell lines which have been started at this institute.

Practical aspects of the successful culture of leukaemia cells include:

(1) The initial cell population should be approximately 4×10^6/ml. and the volume of medium about 50 ml. (depth 4–7 mm) in bottles maintained as stationary cultures or hover cultures because the cells do not adhere to the glass in most instances.

(2) The media used should be supplemented with 20 per cent foetal calf serum or a combination of 10 per cent human serum and 10 per cent foetal calf serum.

(3) Media should be added fractionally (but not replaced) for the first week when required because there is an increase in acidity.

Table 1. CULTURE OF MYELOCYTIC LEUKAEMIA

Initials	Diagnosis*	Date of culture	Interval before growth	Initial medium + serum	R.P.M.I. culture No.
S.K.	AML	2–14–64	46 days	1629 + 30FCS†	6410
E.M.	AML	9–15–64	?	1629 + 30FCS	9154
A.P.	CML	4–20–65	41 days	McCoy's + 20FCS	4265
		(A total of 8 cell lines established at different times)			
M.C.	AML	8–19–65	69 days	1630 + 20FCS	8195
		(A total of 6 cell lines established)			
A.L.	CML	10–19–65	35 days	1640 + 20FCS	1245
N.B.	CML	11–15–65	72 days	1603 + 20FCS	1115
M.J.	AML	12–8–65	47 days	1630 + 20FCS	1285
M.B.	CML	12–10–65	51 days	1630 + 10FCS + 10 Horse	1210

* AML, Acute myelocytic leukaemia. CML, Chronic myelocytic leukaemia.
† FCS, Foetal calf serum. The numbers refer to media developed at Roswell Park Memorial Institute.

(4) The cell population should be maintained above 5×10^5/ml. by combining cultures as necessary.

(5) The cells of the supernatant fluid should be centrifuged and retained and 50–80 per cent of the medium replaced with fresh medium at intervals of 14 days after the sixth week of culture. Fractional additions of media should be made between these intervals.

(6) There should be a rapid feeding and subculturing schedule as soon as the initial restoration of growth is noted. This usually occurs between days 40 and 80.

Characteristics of all the cell lines include a lack of adherence to glass, the formation of clumps shaped like disks or doughnuts, adaptation to suspension cultures, and a predominant morphological appearance of primitive lymphoblasts with smaller numbers of immature myeloid cells. The morphology of the cells, both lymphoblasts and myeloblasts, has been confirmed by electron microscopy.

In two cell lines herpetic-like viruses have been observed.

These cell lines are available to qualified investigators.

G. E. MOORE
E. ITO
P. CITRON

Roswell Park Memorial Institute
(New York State Department of Health),
Buffalo, New York.

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