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Intrathecal Chemotherapy in Burkitt's Lymphoma*

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Summary

Meningeal involvement with Burkitt lymphoma cells constitutes the most challenging therapeutic problem in the management of Burkitt's tumour. The results of intrathecal chemotherapy with methotrexate or cytosine arabinoside in 55 episodes of malignant pleocytosis in 38 patients with Burkitt's tumour are described. The response was complete in nearly all patients after the administration of either agent. Cerebrospinal fluid (C.S.F.) remissions were more prolonged in patients receiving intrathecal methotrexate or cytosine arabinoside daily for four days as opposed to a 10-day schedule. A controlled randomized trial of "prophylactic" intrathecal chemotherapy in patients without malignant cells in the C.S.F. on admission showed no protective effect against the subsequent development of malignant pleocytosis. Future therapeutic approaches are considered in the light of these results.

Introduction

In a previous communication from this centre (Ziegler *et al.*, 1970a) the neurological features of Burkitt's lymphoma were described. The presence of Burkitt lymphoma cells in the cerebrospinal fluid (malignant pleocytosis) was a prominent finding, and though patients with this complication were initially responsive to intrathecal chemotherapy, relapse was common and the prognosis was poor. In a neoplasm characterized by dramatic and often durable remissions following chemotherapy the successful management of malignant pleocytosis presents a major therapeutic challenge (Clifford *et al.*, 1967; Ziegler *et al.*, 1970a). We have now treated 55 episodes of malignant pleocytosis in 38 patients with Burkitt's lymphoma utilizing methotrexate and cytosine arabinoside in seven different intrathecal regimens. This report summarizes the results of intrathecal chemotherapy in this group of patients, some of whom have been followed for up to three years. In addition, prophylactic intrathecal chemotherapy was evaluated in a randomized study in 20 patients with Burkitt's lymphoma presenting without malignant pleocytosis.

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Patients and Methods

All patients admitted to the Lymphoma Treatment Centre, an 18-bed research ward affiliated with Makerere University Medical School, were referred from Mulago Hospital, Kampala, or from up-country district hospitals in Uganda. On arrival at the centre the patients underwent a careful clinical evaluation which included a complete neurological examination and lumbar puncture. The cerebrospinal fluid (C.S.F.) cytology was examined on admission and at least at monthly intervals, several methods (Steel *et al.*, 1965; Ziegler *et al.*, 1970a) being used to detect the presence of malignant cells. The patients were clinically staged according to criteria previously outlined (Ziegler *et al.*, 1970b); these are summarized in Table I.

Previous to August 1969 systemic treatment consisted of intravenous cyclophosphamide, 40 mg/kg, given in one-dose or six-dose schedules (multiple doses given at intervals of two to three weeks) to randomly selected patients who had a complete response following the first dose (Ziegler *et al.*, 1970b). After

TABLE I—*Clinical Staging of Burkitt's Lymphoma*

Stage	Extent of Disease
I	Single facial tumour mass
II	Two or more separate facial tumour masses
III	Intrathoracic, intra-abdominal, paraspinal, or osseous tumour (excluding facial bones)
IV	Central nervous system (malignant cells in the C.S.F.) or bone marrow involvement

the conclusion of this trial, in which multiple doses emerged as the treatment of choice for patients without localized tumours —that is stages III and IV—patients were randomized to six doses of cyclophosphamide versus a sequential regimen consisting of cyclophosphamide (40 mg/kg, intravenously) followed in two weeks by vincristine (1·4 mg/m² intravenously) and methotrexate (15 mg/m² by mouth for four days starting the same day as the vincristine administration), followed in two weeks by cystine arabinoside (250 mg/m² in a daily infusion for three days). The latter sequence was repeated for a total of two cycles. With few exceptions, all patients with malignant pleocytosis were treated systemically with one of the above regimens at the same time that intrathecal chemotherapy was administered.

The different intrathecal chemotherapeutic regimens in the order in which they were used since August 1967 are shown in Table II. A total of 55 episodes of malignant pleocytosis were treated in 38 patients. All patients but two were in the age range of 3 to 14 years. Methotrexate and cytosine arabinoside, diluted in 3 ml of sterile isotonic saline, were instilled with several exchanges of C.S.F. in the syringe. Those receiving methotrexate also had milligramme-equivalent doses of intramuscular citrovorum factor (calcium leucovorin) in six-hourly divided doses over 24 hours and begun at the time of each intrathecal instillation.

All patients with Burkitt's lymphoma presenting between August 1969 and July 1970 who did not have malignant pleocytosis on admission (stages I-III) were randomly allocated to receive either "prophylactic" intrathecal chemotherapy or no intrathecal chemotherapy. The regimen used in patients designated to receive treatment was the four-day alternating schedule of methotrexate and cytosine arabinoside (Table II); two doses of each drug were administered. All randomized patients were subsequently followed with serial lumbar punctures at intervals of two to four weeks.

Results

The results of each intrathecal chemotherapeutic trial are summarized in Table II. All patients but two had complete disappearance of Burkitt lymphoma cells from the C.S.F. within one week following one course of any therapeutic regimen. The two patients who failed to respond completely showed a pronounced reduction but not complete disappearance of malignant pleocytosis following intrathecal chemotherapy. The relapse rate with each regimen was high, however, varying from 60 to 100% (Table II). The mean time to relapse was less than 17 weeks in all instances. Though relapse is defined as the reappearance of Burkitt lymphoma cells in the C.S.F., several patients were noted to relapse with only one to five malignant cells which persisted in the absence of intrathecal therapy for as long as 20 weeks without increasing in number.

It was previously reported that two out of five patients receiving weekly intrathecal methotrexate early in the study achieved long-term C.S.F. remissions (Ziegler *et al.*, 1970a). These patients were still alive and well 144+ and 156+ weeks after therapy. The remaining three patients relapsed with malignant pleocytosis, and subsequent intrathecal therapy with cytosine arabinoside yielded complete, though transient, responses. A subsequent trial using both agents alternately at four-day intervals was evaluated in 15 previously untreated patients; all 13 complete responders eventually relapsed at a median of 10·1 weeks (Ziegler *et al.*, 1970a).

The next trial consisted of methotrexate 15 mg (with citrovorum) given daily for four days to 11 patients, seven of whom had relapsed from the methotrexate and cytosine arabinoside alternate regimen. Eight patients relapsed with a mean remission duration of 16·8 weeks, and the remaining three were well 28+, 39+, and 48+ weeks from therapy.

Six patients relapsing from four-day intrathecal methotrexate and a seventh previously untreated patient were treated with intrathecal cytosine arabinoside, 30 mg daily for four days. Five relapsed on this regimen, with a mean remission duration of 14·4 weeks, and two were still in C.S.F. remission at 9+ and 36+ weeks.

Of the total of 18 patients receiving the four-day regimen, five (four receiving methotrexate and one receiving cytosine arabinoside) had not had previous intrathecal chemotherapy. The relapse rate or C.S.F. remission duration of these five patients was not different from the remainder of patients not previously treated.

In a more aggressive attempt to eradicate malignant cells from the C.S.F. a 10-day schedule of intrathecal methotrexate (with citrovorum) was then instituted. Seven of nine evaluable patients relapsed within four weeks from the completion of therapy (Table II). Five patients previously treated with four-day intrathecal chemotherapy (one receiving methotrexate and four receiving cytosine arabinoside) were not different with respect to relapse rate or remission duration from previously untreated patients.

In a subsequent trial five patients were treated with intrathecal cytosine arabinoside 30 mg, given daily for 10 days. All patients had complete C.S.F. responses, but three relapsed at four, four, and five weeks respectively (Table II), two of whom were previously untreated. The remaining two were well 13+ and 15+ weeks from therapy.

A comparison of the C.S.F. remission duration (life table analysis) observed in patients treated with either four-day or 10-day methotrexate is shown in Fig. 1, and a similar comparison of patients receiving cytosine arabinoside (four-day versus 10-day) is shown in Fig. 2. In the patients receiving methotrexate longer remissions were achieved with the four-day regimen. Both groups were comparable with regard to other clinical variables, including the proportion of patients previously untreated with intrathecal agents. Though the number of patients receiving cytosine arabinoside was small, the remission durations were also longer in those receiving the four-day regimen compared with the 10-day course.

TABLE II—*Intrathecal Chemotherapy of Burkitt's Lymphoma with Methotrexate (MTX) and Cytosine Arabinoside (ARA-C)*

Intrathecal Regimen	No. of Evaluable Patients	No. Receiving Previous Intrathecal Chemotherapy	No. with Complete Response	No. with C.S.F. Relapse	Mean Weeks to Relapse (Range)
*MTX 10 mg weekly	5	0	5	3 (60%)	15·3 (4-27)
ARA-C 10-50 mg weekly	3	3	3	3 (100%)	3·3 (3-4)
*MTX 25 mg alternating with ARA-C 50 mg every 4 days	15	0	13	13 (100%)	10·1 (4-29)
*MTX 15 mg daily × 4	11	7	11	8 (72%)	16·8 (4-43)
ARA-C 30 mg daily × 4	7	6	7	5 (71%)	14·4 (3-32)
*MTX 15 mg daily × 10	9	5	9	7 (78%)	3·4 (2-4)
ARA-C 30 mg daily × 10	5	3	5	3 (60%)	4·3 (4-5)

*With citrovorum factor.

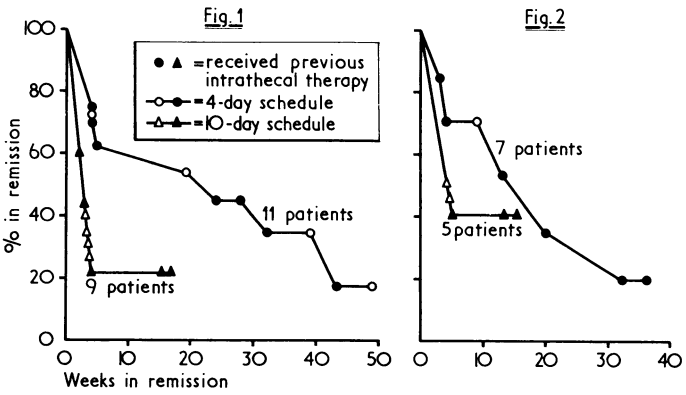

FIG. 1—C.S.F. remission duration in patients receiving intrathecal methotrexate.

FIG. 2—C.S.F. remission duration in patients receiving intrathecal cytosine arabinoside.

An analysis of various factors which might be related to prolonged C.S.F. remissions (> 10 weeks) was carried out. A durable remission was associated with low numbers of malignant cells in the C.S.F. at initiation of therapy, but the correlation was not statistically significant. In addition, patients who developed malignant pleocytosis during their clinical course tended to have more prolonged C.S.F. remissions than those who had this finding on admission. Again, however, this tendency was not statistically significant. No prognostic differences were detected relative to age, sex, site of tumour, presence of concomitant neuropathy or paraplegia, C.S.F. protein value, or mode of concomitant systemic chemotherapy.

Prophylactic intrathecal chemotherapy was evaluated in 20 patients after malignant pleocytosis was excluded by lumbar puncture. Ten were given intrathecal chemotherapy and 10 were not. The results of follow-up in these patients are shown in Table III. Both groups were comparable with respect to age, sex, clinical stage, type of systemic chemotherapy, and duration of observation. Five out of 10 in the treatment group subsequently developed malignant pleocytosis 6, 13, 14, 15, and 15 weeks after initial treatment respectively. Two of these had recurrent tumour masses (one facial, one paraspinal), while in the other three malignant cells in the C.S.F. was the only evidence of the presence of tumour. Four patients in the control group relapsed with malignant pleocytosis 10, 26, 26, and 30 weeks following treatment; two also had simultaneous tumour masses (one facial, one subcutaneous), while two developed malignant pleocytosis alone. This particular regimen of "prophylactic" intrathecal chemotherapy thus appears to offer no protection against the subsequent development of malignant pleocytosis.

TABLE III—*Malignant Pleocytosis Developing in Patients Receiving "Prophylactic" Intrathecal Chemotherapy*

Treatment Group	No. of Patients	No. with Subsequent Malignant Pleocytosis
Intrathecal chemotherapy ..	10	5
No intrathecal chemotherapy ..	10	4

In this prospective study group three patients (one from the treatment group and two from controls) developed only one tumour cell in the C.S.F. This finding was unequivocally confirmed by several observers with a large experience in the cytology of Burkitt's lymphoma. These patients, otherwise in complete remission, have been followed for many weeks without therapy and during this time no increase in C.S.F. cell count has been noted.

Discussion

Central nervous system involvement is a prominent clinical feature in patients with Burkitt's lymphoma, and malignant pleocytosis, encountered in 45% of our patients during the past three years, constitutes our most difficult problem in therapeutic management. In a neoplasm potentially "curable" by chemotherapy a rational and effective approach to the treatment of malignant pleocytosis is critically needed (Ziegler *et al.*, 1970a, 1970b). The pathogenesis of malignant pleocytosis, the kinetics of tumour cells in the C.S.F., and the pharmacology of the cytotoxic agents used are important considerations in this regard.

From recent clinical and pathological studies it appears that Burkitt tumour cells may gain access to the subarachnoid space by direct extension from involved facial bones, cranial or peripheral nerves, or other extradural sites (Clifford *et al.*, 1967; Frank, 1968; Ziegler *et al.*, 1970a). It is possible that tumour cells initially find sanctuary in these extradural sites (particularly perineurium or periosteum) from the effects of systematic cytotoxic agents and subsequently migrate direct to the subarachnoid space much in the manner shown in experimental leukemia in mice (Thomas, 1965). These locations may also preserve cells from the effects of immune cytolysis which is thought to play a part in tumour regressions. The delayed appearance of malignant pleocytosis (often accompanied by cranial neuropathy) in patients otherwise in remission supports this pathogenetic concept. Moreover, there is a significant correlation between patients with these neurological features and the presence of facial or paraspinal tumours (Ziegler *et al.*, 1970a). Malignant pleocytosis may therefore indicate the presence of a "systemic" source of tumour cells either in cranial or peripheral nerves or in clinically occult extradural sites. On a theoretical basis, then, it would be important to administer systemic chemotherapy even when malignant pleocytosis is the only manifestation of disease.

Little is known about the kinetics of malignant cells in the C.S.F. Burkitt lymphoma cells are metabolically active under anaerobic conditions and proliferate rapidly in tissue culture (Cooper *et al.*, 1966). Isotonic, well-circulated C.S.F. should provide sufficient nutrients for cell proliferation. In preliminary studies in our laboratory Burkitt lymphoma cells from the C.S.F. were shown to have a thymidine index of about 30% when incubated with tritiated thymidine in autologous C.S.F. in vitro. This is comparable to the thymidine uptake of Burkitt cells derived from peripheral tumours (Cooper *et al.*, 1966; Iversen *et al.*, 1971). In addition, malignant cells in the C.S.F. are found to contain a thymidine label one hour after the administration of tritiated thymidine intravenously. Preliminary evidence thus indicates that the proliferation kinetics of Burkitt lymphoma cells in the C.S.F. is not substantially different from that of cells in other sites. Recent studies in vitro and in vivo have shown a potential tumour cell doubling time of 24 hours in Burkitt's lymphoma and a high growth fraction (Iversen *et al.*, 1971). If tumour cells in the C.S.F. behave similarly, adequate C.S.F. levels of cycle-active agents such as methotrexate and cytosine arabinoside should be maintained over a period of several doubling times—that is, at least four days—in order to achieve a maximum cytotoxic effect.

The pharmacodynamics of the blood-brain barrier has been reviewed by Rall (1965). With regard to cytotoxic agents administered systemically, only lipid-soluble agents such as the nitrosourea derivatives gain access to the subarachnoid space in appreciable concentration under normal conditions. When the meninges are infiltrated by tumour cells or are inflamed from other causes, however, the permeability of other drugs across the blood-brain barrier may be increased. Bis-chlorethyl nitrosourea has been used in the treatment of Burkitt's lymphoma, but the overall results have been disappointing and formidable toxicity is encountered (Clifford *et al.*, 1967).

Intrathecal chemotherapy and irradiation of the central nervous system has been effective in the treatment of malignant

pleocytosis, and a substantial experience with methotrexate and cytosine arabinoside has been gained in the treatment of meningeal leukaemia. A high percentage of initial complete responses has been reported, but relapse is almost always encountered (Sullivan *et al.*, 1969). The pharmacology of methotrexate administered intrathecally is well characterized (Rall *et al.*, 1962) but little quantitative information is available for cytosine arabinoside. A single intrathecal injection of methotrexate is 99% cleared from the C.S.F. within 24 hours; systemic toxicity can be obviated with systemic citrovorum factor, which does not cross the blood-brain barrier. Though high concentrations of drug can be achieved in the C.S.F., the distribution throughout the subarachnoid space may be variable, and depends to some extent on the volume of administered drug (Reiselbach *et al.*, 1962).

COMPARISON OF REGIMENS

The present study evaluates the comparative effectiveness of different regimens of intrathecal methotrexate and cytosine arabinoside on the response and remission duration of Burkitt lymphoma cells in the C.S.F. Concomitant systemic chemotherapy was administered to nearly all patients, even when malignant pleocytosis was the only manifestation of tumour. The results show that Burkitt lymphoma cells in the C.S.F. are extremely sensitive to the cytotoxic effects of methotrexate and cytosine arabinoside, with only two patients failing to achieve complete clearance of malignant cells from the C.S.F. after one course of any of the regimens used. A comparison of patients receiving methotrexate and cytosine arabinoside on either four-day or 10-day schedules showed longer remission durations in the four-day group. This result was unexpected, as the 10-day regimen was designed as a more aggressive continuous attack on malignant cells in the C.S.F., covering many potential tumour cell doublings.

This observation could be explained (in patients receiving methotrexate) by postulating a tumour-enhancing effect of prolonged administration of citrovorum factor on tumour cells outside the subarachnoid space which were "'seeding" the C.S.F. Alternatively, new tumour cells may have been introduced into the C.S.F. by multiple lumbar punctures, though there was no gross or radiological evidence in any of the patients of paraspinal tumour in the path of the spinal needle. It is also possible that prolonged methotrexate administration arrested the cell cycle outside the S phase with accumulation of cells in G1, which rapidly regained proliferative potential once the drug was stopped (O. S. Selawry and C. G. Zubrod, personal communication). Finally, drug resistance could have developed, though the high concentrations of drug in the C.S.F. should overcome this event (Reiselbach *et al.*, 1963).

Intrathecal cytosine arabinoside appears to be a useful treatment for malignant pleocytosis in Burkitt's lymphoma, and no substantial differences in complete remissions, relapse rate, or remission duration were detected in comparing patients receiving four-day methotrexate with four-day cytosine arabinoside. Further trials of cytosine arabinoside as initial intrathecal therapy will be necessary, as most of the patients treated with this agent had already received previous intrathecal chemotherapy.

MALIGNANT CELLS IN C.S.F.

One important feature which has emerged from these investigations is the achievement in several patients of long-term C.S.F. remissions, indicating the possibility of eradicating malignant cells from the C.S.F. for long periods of time. Another small group of patients developed very low numbers of malignant cells in the C.S.F. for prolonged periods following intrathecal or systemic chemotherapy. Some of these patients eventually develop significant malignant pleocytosis, whereas others are found to have persistently low C.S.F. malignant cell counts. Our initial therapeutic policy was to administer intrathecal chemotherapy to all patients with even one Burkitt lymphoma cell in the C.S.F., but more recently we have been following patients with "indolent" malignant pleocytosis with periodic C.S.F. surveillance. Continued observation will be necessary to determine the clinical significance of this finding. Careful scrutiny of these patients has thus far failed to indicate any predictive or distinctive features.

A randomized trial of prophylactic intrathecal chemotherapy in patients without malignant pleocytosis on admission showed no protective effect against the subsequent development of this complication. This observation implies that tumour cells migrated to the subarachnoid space after initial therapy and were not occultly present in the C.S.F. on admission. The appearance of a simultaneous "systemic" tumour relapse in two of the five treated patients developing malignant pleocytosis supports this concept. Careful surveillance of the C.S.F. in all patients with Burkitt's lymphoma throughout their clinical course is therefore mandatory, and prompt institution of both intrathecal and systemic chemotherapy is indicated when significant malignant pleocytosis is observed.

The results of this study may have important implications for the management of patients with meningeal leukaemia. This complication often develops when the patient is otherwise in complete bone marrow remission. In these patients it may be important to begin systemic induction chemotherapy at the time of meningeal relapse, even though malignant pleocytosis is the only manifestation of disease. Neuropathological evidence indicates that dural deposits are not responsive to intrathecal chemotherapy, whereas arachnoid infiltrations will regress (Wang and Pratt, 1970). Leukaemic cells residing in the meninges but not accessible to intrathecal chemotherapy may thus be reached by cytotoxic agents. The optimum schedule of intrathecal administration is not known, but daily administration for at least four days in a dose and schedule similar to the present study may be useful.

The therapeutic effect of intrathecal administration of a large volume of drug has not been clinically evaluated, but is of obvious anatomical significance with regard to distribution of drug (Reiselbach *et al.*, 1962). The use of the Ommaya reservoir for ventricular perfusion provides a convenient route of administration of intrathecal agents, and may improve drug distribution within the subarachnoid space (Rubin *et al.*, 1966). The dosage, mode of administration, and schedule of intrathecal agents for the management of malignant pleocytosis in acute leukemia and Burkitt's lymphoma now require further critical evaluation in controlled clinical trials.

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ADDENDUM—Since this paper was submitted for publication a randomized therapeutic trial of malignant pleocytosis was conducted, using four daily intrathecal injections of either methotrexate 15 mg or cytosine arabinoside 30 mg administered in 20 ml of Elliott's "B" solution. Ten previously untreated patients entered the trial, and though complete C.S.F. remissions were initially induced, all patients have relapsed. Five received methotrexate and C.S.F. relapse occurred at 3, 4, 6, 8,

and 16 weeks respectively. Five patients received cytosine arabinoside and C.S.F. relapse occurred at 3, 3, 3, 4, and 14 weeks respectively. Thus in this small trial neither agent appears to be superior with regard to remission duration, and the larger volume of diluent did not seem to favourably affect the outcome of these patients compared with our previous experience using a small volume of diluent.

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Leakage of Oxygen from Blood and Water Samples Stored in Plastic and Glass Syringes

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Summary

Theory and experiment showed that samples of blood and water stored in 2-ml and 5-ml syringes made of polypropylene, polystyrene, or S.A.N. co-polymer exchanged oxygen with their surroundings. In the first hour the exchange was due mainly to equilibration with the plastic of the syringe and only in small degree to permeation through the plastic. With high initial tension or with blood of low haemoglobin concentration the exchange can result in errors in Po_2 of up to 6% in two minutes and 16% in 30 to 60 minutes. With all-glass syringes the exchange was much slower but, even so, after 24 hours was important in all but a few of 18 interchangeable glass syringes. Therefore unless analysis can be started immediately all-glass syringes are to be preferred, and for prolonged storage even these should be selected.

Introduction

A fall of oxygen tension in stored blood samples due to metabolism is well recognized. Reports of additional changes in oxygen tension in both blood and water samples stored in plastic syringes are conflicting (Laver and Seifen, 1965; Fletcher and Barber, 1966; Adams *et al.*, 1967), but the work of Hilty and Karendal (1969) leaves no doubt that in some circumstances these additional changes are important. This paper describes work designed to elucidate the mechanism of the changes and to show in what circumstances they are of importance and whether they can be avoided by the use of all-glass syringes.

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Materials and Methods

Oxygen tension was measured with a Radiometer E5046 polarographic oxygen electrode fitted with a 20-μm polypropylene membrane and provided with a digital display of the output current. Zero and sensitivity of the electrode were checked often enough to be able to correct for the small amount of drift which occurred during the experiments. The response of this electrode is linear though the response time may vary. Corrections for incompleteness of response were applied to all measurements (Mapleson *et al.*, 1970).

Sterile distilled water or heparinized fresh blood was equilibrated in bubble tonometers with oxygen at the tension under investigation for one hour at 37°C. The tonometers were sterilized before use in chlorhexidine 1/5,000 to avoid any oxygen consumption by contaminant bacteria. Gas mixtures of known, constant composition were obtained from Wosthoff pumps; mixtures perfusing blood always contained 5% carbon dioxide. The pH and haemoglobin concentration of the blood were determined at the close of each experiment.

Bubble-free samples of water or blood were taken into dry syringes of 2- or 5-ml capacity and aliquots analysed for Po_2 at various time intervals. Between analyses the syringes were sealed with metal or plastic caps and usually stored on the laboratory bench at room temperature. Six makes of commonly used plastic syringe and a single make of interchangeable "all-glass" syringe were investigated. During blood experiments a glass syringe was used as a control to estimate and correct for oxygen consumption by leucocytes (Asmussen and Nielsen, 1961; Hedley-White and Laver, 1964). All experiments were performed by one investigator.

Results

The fall in oxygen tension in water during storage in six different brands of 5-ml plastic syringe and one 5-ml glass syringe is shown in Fig. 1. The water was initially in equilibrium with 96% O_2 and, for comparison with other experiments, the fall in tension is expressed as a percentage of the difference between the initial tension in the water and the oxygen tension in the ambient air. Clearly the fall is considerable for all the plastic syringes and small for the glass syringe.