terial. Neither of these patients had a leucoerythroblastic blood picture, and marrow replacement would not be associated with shortened platelet survival.

Microangiopathic changes were not seen in the peripheral blood smears in either patient. Evidence for a haemolytic anaemia was not observed, nor was there any small-vessel involvement by carcinoma in the patient who came to necropsy. Hence, microangiopathic haemolytic anaemia with thrombocytopenia or a consumption coagulopathy would seem unlikely, but the latter was completely excluded only in Case 1, where the fibrinogen level and thrombin and prothrombin times were normal, and no split products were found. No evidence for any other cause of immune thrombocytopenia was found. Neither patient had been exposed to drugs or chemicals likely to cause thrombocytopenia. L.E. cell preparations were negative, and there was no history of a recent viral infection. Lymphomas and leukaemias were not present.

The fact that neither patient responded to splenectomy and/or steroids does not detract from the diagnosis of thrombocytopenia on an immune basis. Baldini (1966) found in dogs that as the titre of antiplatelet antibodies rises platelets became sequestered in the liver as much as in the spleen and the response to steroids and to splenectomy lessens. Hence, it is postulated that these patients either had a very high titre of antibody or there could be an increase in macrophage activity in the reticuloendothelial system (Firkin *et al.*, 1969). The response to splenectomy or steroids in chronic adult idiopathic thrombocytopenic purpura does not appear to bear any direct relation to the presence or absence of demonstrable splenic sequestration or the degree of reduction of platelet survival (Castaldi and Firkin, 1963; Shulman *et al.*, 1964; Baldini, 1966).

Case 1 showed a very transient rise in platelet count after removal of the tumour. Metastatic carcinoma, however, was present in lymph nodes removed at operation, and it is probable that residual carcinoma remains and could explain the subsequent relapse.

The association of neoplasia with thrombocytopenia may well be due to chance, but it seems important to examine thrombocytopenia in neoplastic states more closely in the future and not to assume that it is due simply to bone marrow replacement by the tumour or a consumption coagulopathy. The possibility that patients in the older age group presenting with thrombocytopenia may have an occult carcinoma should also be remembered by the clinician.

REFERENCES

Baldini, M. (1966). *New England Journal of Medicine*, **274**, 1245, 1301, 1360.

Castaldi, P. A., and Firkin, B. G. (1963). *Australasian Annals of Medicine*, **12**, 333.

Firkin, B. G., Wright, R., Miller, S., and Stokes, E. (1969). *Blood*, **33**, 240.

Harrington, W. J., Minnich, V., and Arimura, G. (1956). *Progress in Hematology*, **1**, 166.

Najean, Y., Ardaillou, N., Caen, J., Larrieu, M. J., and Bernard, J. (1963). *Blood*, **22**, 718.

O'Neill, B., and Firkin, B. G. (1964). *Journal of Laboratory and Clinical Medicine*, **64**, 188.

Shulman, N. R., Marder, V. J., Hiller, M. C., and Collier, E. M. (1964). *Progress in Hematology*, **4**, 222.

Preliminary Communications

Active Immunotherapy with Corynebacterium parvum and Chemotherapy in Murine Fibrosarcomas

British Medical Journal, 1970, **1**, 541–544

Summary: Corynebacterium parvum used alone to enhance immunological reactivity produced transient inhibition of the growth of chemically induced isogenic mouse tumours. Attempts were made to combine C. Parvum with cyclophosphamide to see whether this would increase the latter's effectiveness in inhibiting early but established tumours. Of the various regimens tested, the administration of the C. parvum 12 days after a single dose of chemotherapy produced dramatic inhibition of tumour growth and resulted in complete and lasting regressions in up to 70% of tumour-bearing animals. The most important variable in this regimen is the time between the chemotherapy and the subsequent immunotherapy.

It is possible that non-specific active immunotherapy with agents such as C. parvum may be a valuable adjunct to the conventional cyto-reductive treatments of cancer, but the time of administration of such therapy is probably critical for each tumour and for each chemotherapeutic regimen.

INTRODUCTION

The potential value of immunological methods in the control of cancer has received much attention. It is widely held that though such methods are unlikely to lead to the eradication of extensive malignant disease they may be of value as an adjunct to other therapeutic techniques. There is some experimental evidence to support this suggestion. Haddow and Alexander (1964) demonstrated that rat sarcomas are radiosensitized by autoimmunization of the host with irradiated tumour. Glynn *et al.* (1969) used a combined immunochemotherapeutic system to eradicate Moloney-virus-induced lymphomas in mice. They combined cyclophosphamide with adoptive immunotherapy using tumour-immune lymphocytes and showed that, though neither the chemotherapy nor the immunotherapy used alone was effective, in combination they eliminated the tumours in a significant proportion of treated animals.

Agents which produce a generalized increase in immunological reactivity have been used as a form of non-specific active immunotherapy. Zymosan (Bradner *et al.*, 1958), B.C.G. (Old *et al.*, 1959), and *Corynebacterium parvum* (Woodruff and Boak, 1966) have induced significant host-mediated inhibition of several types of experimental tumour. The exciting results obtained by Mathé (1969) in the treatment of acute lymphoblastic leukaemia in children suggest that such agents may be usefully combined with the more conventional forms of chemotherapy. Sokoloff *et al.* (1961) examined the antitumour activity of mitomycin C and indicated that the oncolysis of several non-specific murine tumours produced by this antibiotic can be enhanced by the use of zymosan. The adjuvant activity of this yeast extract was exquisitely dependent on time of administration, and under some circumstances it merely aggravated the toxic effects of mitomycin C. Martin *et al.* (1964) also used zymosan, combining it with both cyclophosphamide and surgery in the treatment of spontaneous mammary adenocarcinomas in mice. They concluded that zymosan appreciably enhanced the therapeutic effects of the surgery-chemotherapy combination. They also showed that the

timing of administration of zymosan was extremely critical. Heat-killed suspensions of *C. parvum* are highly active non-specific stimulants of both cellular and humoral immunity (Neveu *et al.*, 1964) and show significant antitumour activity when given to mice bearing established tumours (Woodruff and Boak, 1966). This inhibition is transient, however, and death from tumour is not appreciably delayed.

This communication describes experiments designed to determine whether the anti-tumour effects of a conventional alkylating agent can be improved by active immunotherapy. Non-specific active immunotherapy with *C. parvum* was combined with cyclophosphamide in various regimens in an attempt to eradicate established murine fibrosarcomas growing in syngeneic mice.

Materials and Methods

Mice.—The mice used in this study were inbred CBA males about 8 weeks old. They were caged in groups of five and allowed free access to food and water.

Tumours.—Two fibrosarcomas, both induced by 20-methylcholanthrene in CBA male mice, were used in this study. They are moderately well differentiated tumours and possess detectable tumour-specific antigen activity. They were always transplanted by means of a 13-gauge trocar into the shaved right flank of CBA male mice. The first tumour, identified at MC1, had been passaged 20 times before being used. The second tumour, MC6, was used in its first, second, and third passages. The basic experimental design was standardized for all the experiments. Batches of mice were implanted with tumour fragments and then randomly assorted into groups of 10. On the sixth day after inoculation tumour was macroscopically detectable in over 95% of the animals. Those with no detectable nodules at this stage were discarded. Treatment was initiated on the sixth day, when all the treated mice had early but well-established tumours. Groups of 10 control mice were given doses of physiological saline by the appropriate route each time any treatments were administered to the test animals. Maximum tumour diameter was measured three times a week with a transparent millimetre scale.

Cyclophosphamide.—This was given by intraperitoneal injection in 0·2 ml. of saline. After several preliminary pilot experiments the dose chosen for these studies was 200 mg./kg. This dose was well tolerated. Deaths from drug toxicity did not occur.

Corynebacterium parvum.—*C. parvum* strain 10390 was obtained from the National Collection of Type Cultures (Colindale) and cultured as previously described (Currie, 1969). The heat-killed suspension used in these experiments contained about 2 mg. of dry solids per ml. The standard dose per mouse was 200 μg. given intradermally. Previous studies (Currie and Bagshawe, 1969, unpublished) had shown that this route was effective and produced no obvious general toxic reactions. The intradermal injection was made into the opposite flank from the tumour. In some animals the intraperitoneal route was used, but on the whole it was more toxic and generally less effective.

Combination Therapy.—Groups of tumour-bearing mice were treated with cyclophosphamide and/or *C. parvum* from the sixth day onwards in various combinations—that is, giving the *C. parvum* first on Day 6 and then the cyclophosphamide after varying intervals, and vice versa.

Results

Effect of Cyclophosphamide Only.—Cyclophosphamide 200 mg./kg. given on Day 6 produced a profound but transient inhibition of both the tumours studied. The mean diameters are shown in Fig. 1 for MC1 and in Fig. 2 for MC6. Suppression of tumour growth after this single-dose chemotherapy seemed to last about 14 days. This alkylating agent also prolonged the survival of the tumour-bearing mice but produced no complete regressions (See Fig. 3).

Effect of C. parvum Only.—Previous studies (Currie and Bagshawe, 1969, unpublished) had shown that a single injection of *C. parvum* given on Day 6, when the mean tumour diameter was about 2-3 mm., produced a significant depression in the rate of increase of tumour diameter. The present studies confirmed that earlier work. *C. parvum* given as late as Day 18 had a minor effect on tumour growth, which, however, was not statistically significant. No complete regressions were ever obtained with *C. parvum* alone.

Effect of C. parvum and Cyclophosphamide Together.—When the cyclophosphamide and the *C. parvum* were injected on the same day many of the mice became ill with weight loss, diarrhoea, ruffled fur, and hypothermia. This toxicity was seen when the *C. parvum* was given either intradermally or intraperitoneally. Half the mice died before day 20 whereas the remainder went on to die from tumour at about the same time as the controls (see Fig. 4).

C. parvum followed by Cyclophosphamide.—When the *C. parvum* was given first at Day 6 and followed by cyclophosphamide on either Day 9, 12, 18, or 24, there was no apparent additive effect. The survival of such mice did not exceed those treated with cyclophosphamide alone, and this regimen was abandoned.

Cyclophosphamide followed by C. parvum.—*C. parvum* was administered intradermally in a dose of 200 μg./mouse at 3, 6, 10, 12, or 16 days after the cyclophosphamide. This time between the cyclophosphamide and *C. parvum* is referred to as the chemotherapy-immunotherapy (C.I.) interval and the results are shown in Figs. 3 and 4. With lengthening of the C.I. there was a gradual increase in the survival time of the mice. When the C.I. was 12 days the mice bearing MC1 showed dramatic tumour regression. Of the 10 mice in this group only three eventually died of tumour. The remaining seven were alive and well 220 days after inoculation of MC1 and showed no signs of tumour recurrence. All the mice in the control groups and those treated with cyclophosphamide or *C. parvum* alone died of tumour by Day 74. It is apparent from the results (see Fig. 4) that the C.I. is extremely critical. When it was 6 or 16 days no complete regressions occurred. Only at Day 12 was there a significant number of apparent "cures." The effect of this optimal treatment on tumour diameter is seen in Fig. 1 for MC1 and in Fig. 2 for MC6. It can be seen that the addition of this small dose of *C. parvum* produced a considerable additive antitumour effect. The results of treatment of MC6 have not been as promising as those for MC1. Only 30% complete regressions have been obtained so far.

Other modifications of this regimen have been attempted with MC6. Increasing the dose of *C. parvum* had no beneficial effect; it merely increased the toxicity of the combination. Similarly, repeated *C. parvum* administration on alternate days, either intraperitoneally or intradermally, also produced increased toxicity with no further antitumour effect. It seems probable that the optimum C.I. for treatment of MC6 has not yet been obtained or that it is a less antigenic tumour than MC1. More detailed carefully timed studies of this possibility are in progress.

Discussion

These results suggest that *C. parvum* may prove to be a valuable adjunct to chemotherapy in the control of antigenic tumours. Cyclophosphamide alone had a dramatic but temporary effect on both the sarcomas studied. *C. parvum* when used alone had a milder and even more transient tumour inhibitory effect. In no case was either of these agents curative, but when they were combined under the most favourable conditions a substantial number of complete and lasting regressions were obtained. The most crucial variable in using this

Figure 1

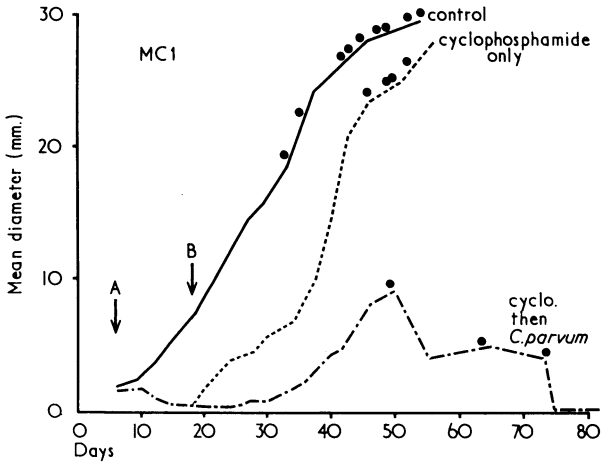

Figure 2

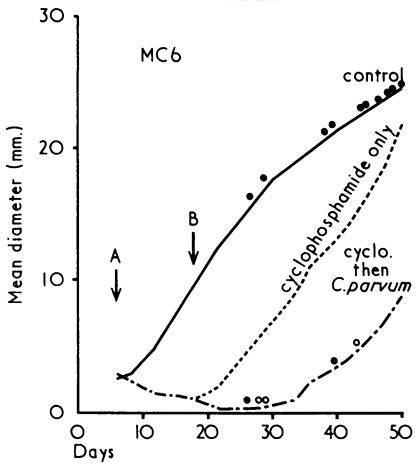

Figure 3

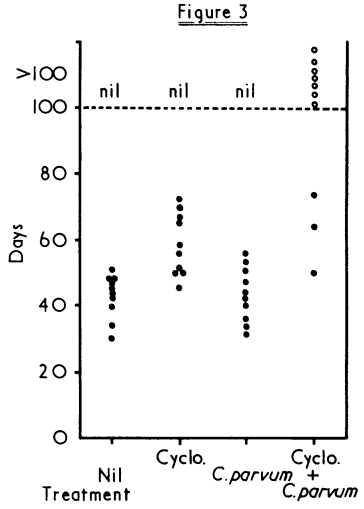

FIG. 1.—Mean diameters of MC1, untreated, treated with 200 mg. of cyclophosphamide per kg., treated with 200 μg. *C. parvum*, and a combination of the two. At point A (Day 6) the mice received cyclophosphamide and at point B they were given intradermal *C. parvum*. Deaths from tumour are denoted as dots. Complete regressions occurred only on the combination treatment. FIG. 2.—Mean diameters of MC6 receiving cyclophosphamide, *C. parvum*, or a combination of both administered as in Fig. 1. The anti-tumour activity of the combination treatment is much greater than that of either the alkylating agent or the immunotherapy; however, only 30% complete regressions were obtained. FIG. 3.—Diagram to show survival of mice bearing MC1, on various treatment regimens. Cyclo=200 mg. cyclophosphamide per kg. given intraperitoneally on Day 6. *C. parvum*=200 μg. *C. parvum* given intradermally on Day 18. Cyclo.+*C. parvum*=Both these treatments given to the same mice. Deaths from tumour are shown as black circles, whereas tumour-free animals are denoted by open circles. The only tumour-free animals surviving over 100 days are those given the combination treatment.

combination seems to be the interval between the single dose chemotherapy and the subsequent non-specific immunological stimulus. When the C.I. interval is zero the combination is extremely toxic with little antitumour activity. This phenomenon may be related to the increased toxicity observed clinically in patients receiving cytotoxic chemotherapy in the presence of infection (Bagshawe *et al.*, 1969).

The use of *C. parvum* as a prelude to the chemotherapy was generally ineffective. When the cyclophosphamide was given first, however, subsequent exposure to *C. parvum* increased the anti-tumour effect. Increasing the C.I. to 10 to 12 days greatly improved the results, with 70% complete and lasting regressions obtained in the treatment of MC1. When the interval was increased to 16 days the tumours readily escaped from the chemotherapy and no regressions were obtained. At the optimum C.I. an increase in the dose of *C. parvum* used was of no further benefit, producing fatal toxic

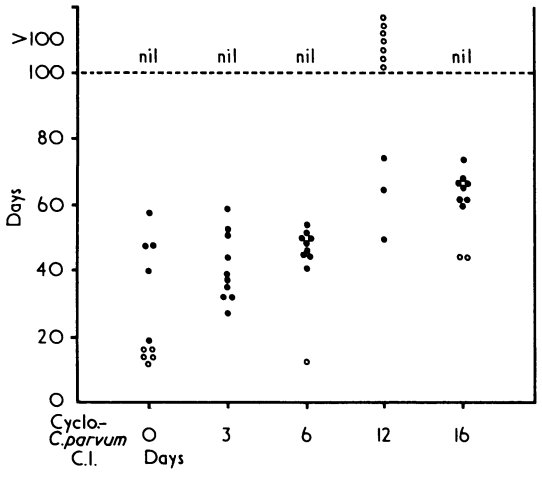

FIG. 4.—Effect on MC1 of varying time interval between the initial cyclophosphamide, given on Day 6, and a subsequent intradermal injection of *S. parvum*. When the chemotherapy-immunotherapy (C.I.) interval is zero the combination is toxic whereas a C.I. of 12 leads to 70% lasting regressions. Black circles denote death from tumour. Open circles denote tumour-free animals.

reactions. Similarly, repeating the dose of *C. parvum* at two-day intervals produced toxicity when started 12 days after cyclophosphamide.

Cyclophosphamide is an effective immunosuppressive agent in the mouse (Fox, 1964) and the importance of the C.I. interval may be related to this effect. It is possible that tumour cell replication and host cellular immune responses escape from the anti-proliferative effects of cyclophosphamide at about the same time. Any stimulant of host cellular responses should be most effective at a time when the tumour content of viable cells is still depleted, but such a stimulant could hardly be expected to operate in the face of severe immunosuppression. Mihich (1969), however, has shown that chemotherapy of the L.1210 leukaemia, though suppressing skin allograft rejection, does not completely inhibit host immune reactions to the leukaemia cells. In general, the host has been confronted by the tumour cells for some time before chemotherapy is started, and the effect of a non-specific immune stimulus would therefore be more likely to involve sensitized lymphoid cells which would be relatively unaffected by the anti-proliferative effects of the chemotherapy. As Mihich (1969) has postulated, during the recovery phase after chemotherapy there is a race between host defences and tumour cell proliferation. It may simply be that the use of an "immunostimulant" such as *C. parvum* gives a head start to the host, but much more complex effects cannot yet be excluded.

The success or failure of this sort of regimen is obviously dependent on a great many variables such as tumour antigenicity, rate of tumour cell replication, immune status of the host, and the type of chemotherapy used. Can active immunotherapy induce regressions when combined with intermittent dose chemotherapy? If so, when must each be administered? Is it possible to design repeating cycles of chemotherapy-immunotherapy which are effective but not lethally toxic? Are specific immunological manœuvres with tumour cells or extracts likely to be as useful as the non-specific "immunity-boosting" techniques? Would combinations of immunotherapy with, say, chemotherapy, surgery, and irradiation be either feasible or effective? These studies indicate that non-specific immunological stimulants are potentially hazardous as well as potentially beneficial. If the use of such agents is to be extended to clinical trials it seems imperative that this should be done only under vigorously controlled conditions. In com-

bination with studies with animal tumours it may then be possible to answer some of the questions posed here and to build a solid foundation of data for the overall assessment of the potential value of active immunotherapy as an adjunct to the more conventional forms of cancer treatment.

One of us (G.A.C.) acknowledges a Research Fellowship from the Wellcome Trust. Dr. J. C. Coleman, of the department of Bacteriology, Fulham Hospital, is thanked for so expertly preparing the *C. parvum* cultures.

G. A. CURRIE,* M.B., M.R.C.P.,
Wellcome Research Fellow, Edgar Laboratory.
K. D. BAGSHAWE, M.D., F.R.C.P.,
Charing Cross Group of Hospitals, Consultant Physician.
Fulham Hospital, London W.6.

*Present appointment: Member of Scientific Staff, Chester Beatty Research Institute, Belmont, Sutton, Surrey.

REFERENCES

Bagshawe, K. D., Golding, P. R., and Orr, A. H. (1969). *British Medical Journal*, **3**, 733.
Bradner, W. T., Clarke, D. A., and Stock, C. C. (1958). *Cancer Research*, **18**, 347.
Currie, G. A. (1969). In *Foetal Autonomy*, edited by M. O'Conor, and G. E. Wolstenholme, p. 32. London, Churchill.
Fox, M. (1964). *Transplantation*, **2**, 475.
Glynn, J. P., Halpern, B. L., and Fefer, A. (1969). *Cancer Research*, **29**, 515.
Haddow, A., and Alexander, P. (1964). *Lancet*, **1**, 452.
Martin, D. S., Hayworth, P., Fugmann, R. A., English, R., and McNeill, H. W. (1964). *Cancer Research*, **24**, 652.
Mathé, G. (1969). *British Medical Journal*, **4**, 7.
Mihich, E. (1969). *Cancer Research*, **29**, 848.
Neveu, T., Branellec, A., and Biozzi, G. (1964). *Annales de l'Institut Pasteur*, **106**, 771.
Old, L. J., Clarke, D. A., and Benacerraf, B. (1959). *Nature*, **184**, 291.
Sokoloff, F., *et al.* (1961). *Growth*, **25**, 249.
Woodruff, M. F. A., and Boak, J. L. (1966). *British Journal of Cancer*, **20**, 345

Medical Memoranda

Portal Hypertension Presenting with Haemoperitoneum

British Medical Journal, 1970, **1**, 544

The most serious complication of portal hypertension is massive haemorrhage from varicose portal-systemic venous anastomoses. The site of bleeding is almost invariably the gastro-oesophageal varices.

This report describes a case of portal hypertension which presented with a massive intraperitoneal haemorrhage from a ruptured paraumbilical varix. It is believed to be the only such case reported.

CASE REPORT

A Spanish-speaking Gibraltarian, aged 38, who had recently arrived in England had no significant past history and had been in good health up to the day of admission to hospital. Five hours before his admission he had suddenly developed a lower abdominal pain. He vomited several times and had had his bowels open five times in as many hours, but there was no blood in either his vomitus or faeces. On examination he was shocked, the systolic blood pressure was 60 mm. Hg, and the pulse rate 130. When lying flat, he had pain in both shoulders. Free fluid was present in the abdomen and its volume was noted to increase while he was being resuscitated. A diagnosis of haemoperitoneum was made and he was taken to the theatre after the rapid infusion of plasma expanders and whole blood had been instituted.

Through a right paramedian incision the peritoneal cavity was opened and was found to contain 6 pints (3·4 litres) of blood. The liver was about three-quarters normal size and was uniformly nodular; a liver biopsy was subsequently reported to show portal cirrhosis. The spleen was enlarged to about six times the normal size. After a prolonged search the bleeding point was found to be a ruptured paraumbilical varix, which was then oversewn.

Postoperatively he made a satisfactory recovery except for a significant ooze of blood from his wound which persisted for two days. He was found to have glycosuria and required eight units of soluble insulin twice daily to keep his blood sugar within normal limits. He was noted to have liver palms but no spider naevi.

He was investigated with a view to carrying out definitive treatment of his portal hypertension. Many investigations were carried out, but only those that were abnormal are recorded here: prothrombin time was twice the control value and did not alter significantly despite daily vitamin-K injections, fibrinogen 115 mg., total bilirubin 1·9 mg., serum aspartate aminotransferase 266 units, serum alanine aminotransferase 135 units, but 10 days after operation the transaminases had fallen to 36 and 37 units respectively. Barium swallow demonstrated small oesophageal varices. Subsequently, he was referred to another hospital for consideration of elective portacaval anastomosis.

COMMENT

Child (1964) stated that bleeding associated with portal hypertension occurs "rarely if ever" from sites other than oesophageal varices but Milnes Walker (1959) included two case reports from the literature of haemorrhage from other sites.

Levy, Hardin, Shipp, and Keeling (1957) reported a case of portal hypertension presenting with gastrointestinal haemorrhage which was localized to varices in the region of the caecum. Hermann and Esselstyn (1967) reported the case of a girl who bled per rectum from varices in the sigmoid colon.

A case of portal hypertension presenting with haemoperitoneum was described by Ellis, Griffiths, and MacIntyre (1958). At necropsy the source of bleeding was found to be a cluster of varices in front of the right kidney, and the resulting haematoma had ruptured into the peritoneal cavity. These authors reviewed 129 cases of haemoperitoneum, of which only the case mentioned above was the result of ruptured varices in association with portal hypertension. It is interesting to note that Armstrong, Adams, Tragerman, and Townsend (1942) analysed 55 cases of the Cruveilhier–Baumgarten syndrome and, though 10 cases developed haematemeses, haemoperitoneum was not recorded in any.

My thanks are due to Mr. N. E. Stidolph for permission to publish details of this case.

A. P. ROSS, M.S., F.R.C.S.
London, N.6.

REFERENCES

Armstrong, E. L., Adams, W. L., Tragerman, L. J., and Townsend, E. W. (1942). *Annals of Internal Medicine*, **16**, 113.
Child, C. G. (1964). *The Liver and Portal Hypertension*. Philadelphia, Saunders.
Ellis, H., Griffiths, P. W. W., and MacIntyre, A. (1958). *British Journal of Surgery*, **45**, 606.
Hermann, R. E., and Esselstyn, C. B. (1967). *Archives of Surgery*, **95**, 956.
Levy, J. S., Hardin, J. H., Shipp, H., and Keeling, J. H. (1957). *Gastroenterology*, **33**, 637.
Walker, R. M. (1959). *The Pathology and Management of Portal Hypertension*. London, Arnold.