Using direct immunofluorescence to detect coronaviruses in peritoneal and pleural effusions

M. Cammarata Parodi, G. Cammarata, S. Paltrinieri*, A. Lavazza† and F. Ape

Istituto di Anatomia Patologica Veterinaria e Patologia Aviare, Via Celoria 10, 20133 Milano, Italy, *Istituto di Patologia Generale Veterinaria, Milano, Italy and †Istituto Zooprofilattico Sperimentale, Brescia, Italy

Journal of Small Animal Practice (1993) **34**, 609-613

ABSTRACT

Twenty-one cases of feline infectious peritonitis (FIP) were diagnosed using a direct immunofluorescence test on cytocentrifuged pleural and peritoneal effusions from cats sampled in vivo (11 cases) and at necropsy (10 cases). A commercial fluorescent polyclonal antiserum of feline origin reacting with FIPV and cross reacting with transmissible gastroenteritis virus and canine coronavirus was used. Eleven cats with ascites of a different origin were used as negative controls. The direct immunofluorescence test was 97 per cent reliable (31 cases of 32) and can be used in routine diagnosis.

INTRODUCTION

Feline infectious peritonitis (FIP) is a disease which affects domestic cats and several wild feline species (Barlough and Weiss 1983, Pedersen 1987a, Scott 1987, Barlough and Stoddart 1990). It was described for the first time in the USA by Wolfe and Griesemer (1966) and is now reported worldwide.

FIP is caused by a virus of the Coronaviridae family, antigenically related to other coronaviruses which are responsible for mild feline infections, such as feline enteric coronavirus (FeCV), or asymptomatic infections, like transmissible gastroenteritis virus (TGEV) or canine coronavirus (CCV) (Reynolds and others 1977, Pedersen and others 1978, Holmes 1985, McIntosh 1985, Fenner and others 1987, Tupper and others 1987, Pastoret and Burtonboy 1991).

FIP is not easy to diagnose in vivo, partly because of its subtle onset and partly because of the variety of signs and lesions which accompany the different clinical forms (Doherty 1971, Wolfe and Griesemer 1971, Montali and Strandberg 1972, Pastoret and others 1974, Legendre and Whitenack 1975, Hayashi and others 1977, Rosmini and Simoni 1979, Weiss and Scott 1981, Pedersen 1983b, Barlough and Summers 1984, Lutz and others 1985, Renzoni and others 1985a,b, Pedersen 1987a,b, Kelnerr and Litschi 1989). Suspicion of the onset of peritoneal forms is often aroused by the appearance of ascites, accompanied by listlessness, mild jaundice, and fever, which does not respond to antibiotic treatment.

Diagnosis is much more difficult in effusive non-peritoneal forms and even more so in non-effusive forms (Robison and others 1971, Lutz and others 1985, Pozza and Avezza 1986, Pedersen 1987a,b, Wise and Macy 1990).

The titration of circulating anti-FIPV antibodies is not diagnostic, although reliable results are often obtained using the enzyme-linked immunosorbent assay (ELISA) or competitive ELISA (C-ELISA) (Faravelli and others 1991). In fact FIP signs appear only in a proportion of seropositive felines and in some cases antibody levels become undetectable during the course of the disease. False positives due to infections from serologically related coronaviruses are also reported (Pedersen and others 1980, Weiss and Scott 1980, Pedersen 1983a,b, Barlough 1985, Pedersen 1987b, Barlough and Stoddart 1990, Wise and Macy 1990).

According to Shelly and others (1988), physicochemical examination of the intracavitary effusions can supply useful information, especially when the γ-globulin concentration exceeds 32 per cent. At the moment it appears that there are

Table 1. Comparison between the pathological findings and results of the direct immunofluorescence (DIF) test on effusions from 32 cats

Number	Breed	Sex	Age	Effusion	Sample	Final diagnosis	DIF
1	Persian	M	6 months	Peritoneal	pm	FIP	+
2	Persian	F	6 months	Peritoneal	am	FIP	+
3	Persian	F	6 months	Peritoneal	pm	FIP	+
4	ND	ND	ND	Pleural	am	FIP	+
5	Mongrel	F	3 years	Peritoneal	am	Nocardiosis	–
6	Mongrel	F	2 years	Pleural	am	Nocardiosis	–
7	Persian	M	3 months	Peritoneal	pm	FIP	+
8	Persian	F	6 months	Peritoneal	am	FIP	+
9	Persian	F	1 year	Peritoneal	am	FIP	+
10	Persian	F	3 months	Peritoneal	am	FIP	+
11	Persian	M	4-8 months	Peritoneal/pleural	am/pm	FIP	–
12	Mongrel	F	6 years	Peritoneal	pm	Hepatodystrophy	–
13	Mongrel	M	5 years	Peritoneal	pm	FIP	+
14	Mongrel	F	10 years	Pleural	am	Chylothorax	–
15	Mongrel	M	2 years	Peritoneal	am	FIP	+
16	Mongrel	F	2 years	Pleural	am	Mesothelioma	–
17	Mongrel	F	5 years	Peritoneal	am	FIP	+
18	Mongrel	F	7 months	Peritoneal	am	FIP	+
19	Mongrel	F	2 years	Peritoneal	pm	FIP	+
20	Mongrel	M	3 years	Peritoneal/pleural	am/pm	FIP	+
21	Mongrel	F	3 years	Peritoneal	am	Hepatodystrophy	–
22	Mongrel	M	2 years	Peritoneal	pm	FIP	+
23	Mongrel	F	2 years	Peritoneal/pleural	pm	FIP	+
24	Mongrel	M	ND	Peritoneal	pm	FIP	+
25	ND	ND	ND	Peritoneal	am	Foreign body peritonitis	–
26	Persian	F	3 months	Peritoneal/pleural	am	FIP	+
27	Persian	F	2 months	Peritoneal	am	Nocardiosis	–
28	Mongrel	M	14 years	Pleural	am	Pulmonary carcinoma	–
29	Mongrel	F	4 months	Peritoneal	am/pm	FIP	+
30	ND	ND	ND	Pleural	am	Septic pleurisy	–
31	Mongrel	M	1 year	Pleural	pm	Mesothelioma	–
32	ND	ND	ND	Peritoneal	am	FIP	+

am Ante mortem examination, pm Post mortem examination, ND Not determined, FIP Feline infectious peritonitis

no suitable techniques for the direct demonstration of FIPV in such effusions.

In order to overcome these difficulties the authors tried to evaluate the results of direct immunofluorescence (DIF) on cytocentrifuged cavitary effusions of affected animals in comparison with cryostatic sections of the related organs. The aim was to verify the possible application of this method to intravital diagnosis of FIP.

MATERIALS AND METHODS

Thirty-two cats were included in the investigation. They all showed signs of FIP. In particular, signs of effusions in at least one serous cavity were present.

Approximately 2 ml of effusive fluid were sampled in vivo from the affected cavity of 22 cats. In the remaining 10 cats, sampling of effusive fluid was carried out at necropsy within two days of death.

The information regarding sex, breed and age of the cats is shown in Table 1.

Within 15 hours of sampling, two slides were obtained from each sample through cytocentrifugation using the Cytospin 2 (Shandon) at 130 *g* for 10 minutes. One slide was stained with May Grünwald-Giemsa and the other submitted for DIF.

All the cats, including those sampled in vivo, were subjected to post mortem examination. The final diagnosis was based on the necropsy findings and histological examination.

A commercial feline polyclonal fluorescein-conjugated antiserum (VMRD Inc) was chosen for the DIF test; this detects both FIPV biotypes I and II, and cross reacts with TGEV and CCV.

The test was applied on freshly prepared cytocentrifugates and cryostatic sections of organs with typical FIP lesions (10 cases). The control sections came from organs with lesions from other diseases (11 cases). If immediate staining was not possible, it was carried out after the slide had been stored at –20°C for no more than seven days.

The slides submitted for DIF were fixed and

dehydrated in acetone-methanol (75 to 25 per cent) for 20 minutes and incubated with 100 µl of labelled serum for 30 minutes at 37°C in a moist chamber. After washing four times for 10 minutes with a 25 per cent solution of carbonate buffer (pH 9), the slides were mounted with buffered glycerol and examined under a fluorescent microscope at 250 to 400 × magnification. The technique was verified using a known positive cryostatic section.

An attempt was made to show the presence of coronavirus by transmission electron microscopy on 10 samples of ascitic fluid chosen from those cats positive to the DIF, and following ultracentrifugation with a Beckman Airfuge and negative staining with a 2 per cent sodium salt of phosphotungstic acid.

RESULTS

A clear correlation was found between pathological findings and analysis of the intracavitary effusions by DIF for all the cats examined, except one (Table 1).

In 11 of the 32 cats, the pathological picture and laboratory tests led to a diagnosis different to FIP, referable to nocardiosis (three cases), intrathoracic neoplasms (three cases), hepatodystrophy (two cases), foreign body peritonitis (one case), septic pleurisy (one case), and chylothorax (one case). In all of these cases the cytocentrifugates of the intracavitary effusions were negative by the DIF test. May Grünwald-Giemsa stain often supplied useful indications for the diagnosis, which were subsequently confirmed by histological and, or, microbiological examination.

In the remaining 21 cats, the clinical diagnosis of FIP was confirmed by pathological and histological findings and was also confirmed in 10 of these cases by a positive DIF test carried out on cryostatic sections of affected organs.

A marked disagreement between the result from the DIF test on ascitic fluid and the final FIP diagnosis was found in only one case (case 11; Table 1) which at the age of four months showed clinical signs of thoracic effusions with fever. Both the ELISA for the detection of anti-FIPV antibodies and DIF test carried out on the effusions were negative. Following antibiotic-cortisone treatment, the clinical signs partially subsided and general health improved, with the exception of persistent fever. After four months of treatment, there was a sudden serious deterioration in the clinical picture and concurrent appearance of ascites and serious cardiac failure. Further ELISAs gave a positive result for FIP whereas the DIF test on peritoneal fluid remained negative. Shortly before death, the ELISA gave a negative or doubtful result and the DIF test on the exudate again gave a negative result. Nevertheless, the histopathological examination and the DIF test, repeated on cryostatic sections of the damaged organs, supported the final diagnosis of FIP.

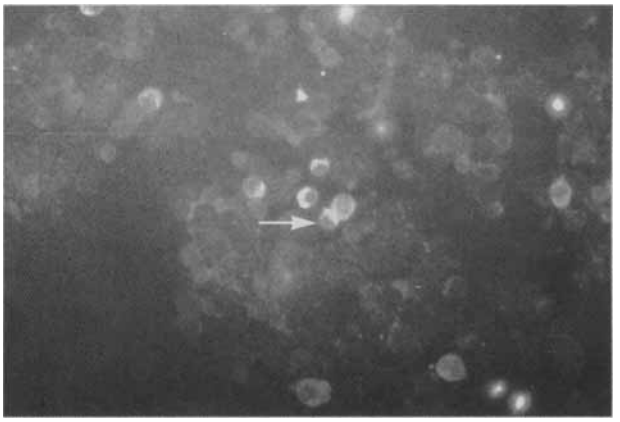

FIG 1. Direct immunofluorescence on a cytocentrifuged peritoneal effusion from a six-month-old female Persian cat with effusive feline infectious peritonitis. The positive cells show a vivid green cytoplasmic fluorescence (arrow). × 400

In the positive exudates the examination of cytocentrifugates by ultraviolet microscope showed variable numbers of cells with a vivid green cytoplasmic fluorescence (Fig 1). Granulocytes also showed a green cytoplasmic fluorescence, similar to that of the positive elements, although they were easily recognisable by the plurilobated nucleus. The autofluorescence of other cells was also different from that of positive cells because it was not so intense and the colour tended more towards yellow.

The corresponding cytocentrifugates stained by the May Grünwald-Giemsa method showed pictures consistent with FIP infiltrates: there was a polymorphous cell population mainly composed of macrophages, lymphocytes, mesothelial cells and occasionally granulocytes.

The result of the DIF test was verified on cryostatic sections prepared from affected organs. They showed cytoplasmic fluorescence in mononucleate round cells which infiltrated the necrotic areas.

However, the DIF-positive cells were represented in a different fashion both in mononuclear infiltrates and in various affected organs. So in the same animal it was possible to find organs with positive lesions and organs with negative lesions, and often the mononuclear infiltrates revealed the presence of coronavirus antigen in a limited number of macrophages only.

On ultramicroscopic examination, the presence of coronavirus particles in the intracavitary effusions positive to the DIF test was confirmed in five of the 10 samples examined. Most of the viral particles appeared under the form of immunocomplexes, that is compact clumps of a

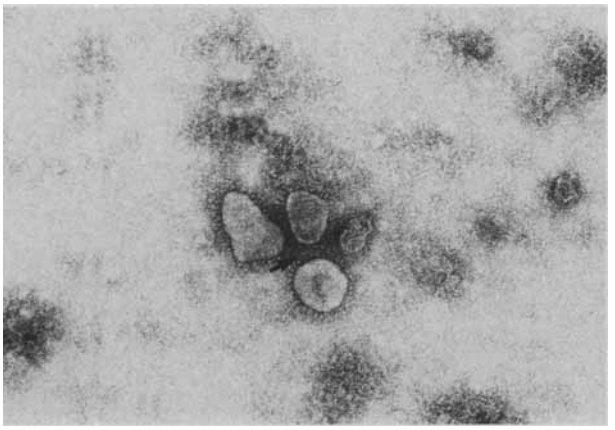

FIG 2. Negative staining of an ultracentrifugate from the peritoneal effusion of a one-year-old male Persian cat with effusive feline infectious peritonitis. The viral particles are coronaviruses. × 65,000

variable number of particles embedded in an electron dense matrix (Fig 2).

DISCUSSION

It is well known that the clinical diagnosis of FIP is frequently difficult (Robison and others 1971, Pedersen 1983b, Lutz and others 1985, Pozza and Avezza 1986, Pedersen 1987a,b, Shelly and others 1988); this also emerged from the authors' experience and particularly from the regular occurrence of suspected cases, not confirmed at necropsy or by laboratory tests. This was occasionally observed even when the clinical history was suggestive and serological tests were positive.

Similar conclusions regarding the reliability of the serological test had already been drawn by other authors (Weiss and Scott 1980, Tupper and others 1987, Ingersoll and Wylie 1988a,b, Barlough and Stoddart 1990, Wise and Macy 1990). It is well known that on the basis of this test alone there are no differences between cats that are clinically ill with FIP and those that are either only infected or have come into brief contact with the virus or have been infected with FeCV. On the other hand, the absence of serum antibodies does not mean exclusion of infection, because the formation and deposition of immunocomplexes can cause temporary 'antibody eclipses' (Pedersen and others 1978, 1980, Pedersen 1987b).

The DIF test that the present authors used on cytocentrifugates from intracavitary effusions is very suitable, giving a positive result in most of the cases of FIP (20 out of 21) which were subsequently confirmed by necropsy and histopathological examinations and, or, by a DIF test on cryostatic sections.

The cases where pathological entities different to FIP were identified and where the DIF test had never been positive on either the samples of the effusions or the cryostatic sections of affected organs were useful negative controls.

However, the single case of FIP where the DIF on the intracavitary effusion was negative should not be underestimated. Therefore, these results seem to suggest that a positive DIF test on the intracavitary fluids can be considered reliable for the diagnosis of FIP, whereas the negative results are less reliable, due to false negatives, even though these are a rare occurrence.

Various authors (Weiss and Scott 1980, Barlough and Stoddart 1990) state that the most reliable diagnostic method consists of a histological biopsy examination: by using the DIF test on the ascitic liquid, biopsy could be limited to those cases where non-effusive FIP disease is suspected.

REFERENCES

BARLOUGH, J. E. (1985) Cats, coronaviruses and coronavirus antibody tests. *Journal of Small Animal Practice* **26**, 353-362

BARLOUGH, J. E. & STODDART, C. A. (1990) Feline infectious peritonitis. *Veterinary Reports* **1**, 13-17

BARLOUGH, J. E. & SUMMERS, B. A. (1984) Encephalitis due to feline infectious peritonitis virus in a twelve-week-old kitten. *Feline Practice* **14**, 43-46

BARLOUGH, J. E. & WEISS, R. C. (1983) Viral diseases: feline infectious peritonitis. In: Current Veterinary Therapy VIII, Small Animal Practice. Ed R. W. Kirk. W. B. Saunders, Philadelphia. pp 1186-1193

DOHERTY, M. J. (1971) Ocular manifestation of feline infectious peritonitis. *Journal of the American Veterinary Medical Association* **159**, 417-424

FARAVELLI, G., PELLINI, P., GILARDINI, R. & AVEZZA, F. (1991) Ricerca degli anticorpi anti-FIPV in gatti sani e malati mediante tecnica immuno-enzimatica competitiva (C-ELISA). *Summa VII*, 148-149

FENNER, F., BACHMANN, P. A., GIBBS, E. P., MURPHY, F. A., STUDDERT, M. J. & WHITE, D. O. (1987) Coronaviridae. In: Veterinary Virology. Academic Press, London. pp 505-518

HAYASHI, T., GOTO, N., TAKAHASHI, R. & FUJIWARA, K. (1977) Systemic vascular lesions in feline infectious peritonitis. *Japanese Journal of Veterinary Science* **39**, 365-377

HOLMES, K. V. (1985) Replication of coronaviruses. In: Virology. Ed B. N. Fields. Raven Press, New York. pp 1331-1343

INGERSOLL, J. D. & WYLIE, D. E. (1988a) Identification of viral antigens that induce antibody responses on exposure to coronaviruses. *American Journal of Veterinary Research* **49**, 1467-1471

INGERSOLL, J. D. & WYLIE, D. E. (1988b) Comparison of serologic assays for measurement of antibody response to coronavirus in cats. *American Journal of Veterinary Research* **49**, 1472-1479

KELNERR, S. J. & LITSCHI, B. (1989) Augenveranderungen bei der felinen infektiosen peritonitis. *Kleintierpraxis* **34**, 261-266

LEGENDRE, A. M. & WHITENACK, D. L. (1975) Feline infectious peritonitis with spinal cord involvement in two cats. *Journal of the American Veterinary Medical Association* **167**, 931-932

LUTZ, H., HAUSER, B. & HORZINEK, M. C. (1985) La peritonite infectieuse feline: etat actuel de connaisances. *Annales de Médicine Veterinaire* **129**, 459-470

McINTOSH, K. (1985) Coronaviruses. In: Virology. Ed B. N. Fields. Raven Press, New York. pp 1323-1330

MONTALI, R. J. & STRANDBERG, J. D. (1972) Extraperitoneal lesions in feline infectious peritonitis. *Veterinary Pathology* **9**, 109-121

PASTORET, P. P. & BURTONBOY, S. (1991) Le point sur la peritonite infectieuse feline. *Annales de Médicine Veterinaire* **135**, 35-41

PASTORET, P. P., GOUFFAUX, M. & HENROTEAUX, M. (1974) Description et etude experimentale de la peritonite infectieuse feline. *Annales de Médicine Veterinaire* **118**, 479-492

PEDERSEN, N. C. (1983a) Feline infectious peritonitis and feline enteric coronavirus infections. Part I: Feline enteric coronavirus. *Feline Practice* **13**, 5-13

PEDERSEN, N. C. (1983b) Feline infectious peritonitis and feline enteric coronavirus infections. Part II: Feline infectious peritonitis. *Feline Practice* **13**, 13-19

PEDERSEN, N. C. (1987a) Coronavirus diseases (coronavirus enteritis, feline infectious peritonitis) In: Diseases of the Cat. Ed H. J. Holzworth. W. B. Saunders, Philadelphia. pp 193-210

PEDERSEN, N. C. (1987b) Virologic and immunologic aspects of feline infectious peritonitis virus infection. *Advances in Experimental Medicine and Biology* **218**, 529-550

PEDERSEN, N. C., BOYLE, J. F., FLOYD, K., FUDGE, A. & BARKER, J. (1980) An enteric coronavirus infection of cats and its relationship to feline infectious peritonitis. *American Journal of Veterinary Research* **42**, 368-377

PEDERSEN, N. C., WARD, J. & MENGELING, W. L. (1978) Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species. *Archives of Virology* **58**, 45-53

POZZA, O. & AVEZZA, F. (1986) Feline infectious peritonitis. *Bollettino Associazione Italiana Veterinari Piccoli Animali* **25**, 7-19

RENZONI, G., TACCINI, E. & BRACA, G. (1985a) Osservazioni anatomo-istopatologiche in corso di peritonite infettiva del gatto e rilievi patogenetici. *Atti Societa' Italiana delle Scienze Veterinarie* **39**, 555-557

RENZONI, G., TACCINI, E. & BRACA, G. (1985b) Ulteriori rilievi sulla peritonite infettiva del gatto (FIP). *Annali della Facolta di Medicina Veterinaria Pisa* **XXXVIII**, 395-407

REYNOLDS, D. L., GARWES, D. J. & GASKELL, C. J. (1977) Detection of transmissible gastroenteritis virus neutralizing antibody in cats. *Archives of Virology* **55**, 77-86

ROBISON, R. L., HOLZWORTH, J. & GILMORE, C. E. (1971) Naturally occurring feline infectious peritonitis: signs and clinical diagnosis. *Journal of the American Veterinary Medical Association* **158**, 981-986

ROSMINI, R. & SIMONI, P. (1979) Peritonite infettiva dei gatti: contributo anatomopatologico ed ultrastrutturale. *Atti Societa' Italiana delle Scienze Veterinarie* **33**, 249

SCOTT, F. W. (1987) Feline coronaviruses: which are really pathogenic? *Le Point Veterinaire* **19**, 191-197

SHELLY, S. M., SCARLETT-KRANZ, J. & BLUE, J. T. (1988) Protein electrophoresis on effusion from cats as a diagnostic test for feline infectious peritonitis. *Journal of the American Animal Hospital Association* **24**, 495-500

TUPPER, G. T., EVERMANN, J. F., RUSSELL, R. G. & THOULESS, M. E. (1987) Antigenic and biological diversity of feline coronaviruses: feline infectious peritonitis and feline enteritis virus. *Archives of Virology* **96**, 29-38

WEISS, R. C. & SCOTT, F. W. (1980) Laboratory diagnosis of feline infectious peritonitis. *Feline Practice* **10**, 16-22

WEISS, R. C. & SCOTT, F. W. (1981) Pathogenesis of feline infectious peritonitis: pathologic changes and immunofluorescence. *American Journal of Veterinary Research* **42**, 2036-2047

WISE, L. A. & MACY, D. W. (1990) Immunodiagnosis of feline infectious diseases. *Compendium on Continuing Education for the Practicing Veterinarian* **12**, 501-511

WOLFE, L. G. & GRIESEMER, R. A. (1966) Feline infectious peritonitis. *Pathologia Veterinaria* **3**, 255-270

WOLFE, L. G. & GRIESEMER, R. A. (1971) Feline infectious peritonitis: review of gross and histopathologic lesions. *Journal of the American Veterinary Medical Association* **158**, 987-993

ABSTRACTS

Infiltrative lipoma in a canine stifle joint

A 10-year-old spayed lhaso apso had a slowly enlarging mass on the right hindleg, extending from the distal femur to mid tibia. This had been present for three years. There was recent lameness in the affected limb. Radiographs demonstrated soft tissue swelling with periosteal new bone on the tibia, and lytic lesions on the tarsus and tibia. Aspirants of the mass were consistent with a diagnosis of lipoma. An infiltrative fatty tumour was found on surgical exploration, involving both soft tissue and bone and extending into, and throughout, the stifle joint. Histological examination confirmed this to be a lipoma. No other treatment was undertaken and the mass continued to enlarge and was only mildly painful. A second biopsy confirmed the mass as remaining lipomatous.

FRAZIER, K. S., HERRON, A. J., DEE, J. F. & ALTMAN, N. H. (1993) *Journal of the American Animal Hospital Association* **29**, 81-83

Chronic vaginal prolapse during pregnancy in a bitch

A FOUR-year-old labrador bitch had a chronic vaginal prolapse, first noticed during oestrus. Artificial insemination had been performed after the prolapse had been manually reduced. Multiple prolapses, treated unsuccessfully by sutures, recurred in the ensuing weeks (up to 58 days post oestrus). Radiographic examination failed to demonstrate any fetal skeletons and the bitch was deemed not to be pregnant. Surgical correction by hysteropexy and surgical removal of the prolapse took place and recovery was uneventful. Six days later, the bitch delivered a live, healthy male pup. Serum progesterone and oestradiol concentrations were taken and were 1·8 ng/ml and 1·75 pg/ml, respectively. Bitches with chronic vaginal prolapse should not be used for breeding.

MEMMON, M. A., PAVLETIC, M. M. & KUMAR, M. S. A. (1993) *Journal of the American Veterinary Medical Association* **202**, 291-295

BOOKS RECEIVED

Keeping British Birds

By F. Meaden. Published by Blandford Press, London. Price £15.99. Paperback. 320 pages. 1993.