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PRODUCTION OF 19S AND 7S ANTIBODIES BY CANCER PATIENTS

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SUMMARY

The production of 7S and 19S antibody was compared in twenty-one patients with various types of carcinoma, four with lymphomatous neoplasms, and eleven healthy controls, at weekly intervals following administration of 17D yellow fever live virus vaccine. Sera were also tested for interferon and infectious virus. Mean and range of antibody titres were very similar in carcinoma patients and controls. There was a slight delay in antibody production in the cancer patients. Viraemia was demonstrated in 1/8 healthy controls, 4/20 carcinoma patients, and 2/3 lymphoma patients. No interferon was detected.

INTRODUCTION

Production of serum antibodies is depressed in many patients with lymphosarcoma, lymphatic leukaemia and multiple myeloma, but patients with other types of cancer, including Hodgkin's disease, usually produce normal levels of serum antibodies (Southam & Moore, 1954; Southam & Green, 1958; Aisenberg & Leskowitz 1963; Cone & Uhr, 1964; Larson & Tomlinson, 1953). However, there is at least one report (Lytton, Hughes & Fulthorpe, 1964) of impaired antibody formation by patients with advanced carcinoma.

Impairment of the delayed type of immunologic responses as shown by delayed rejection of homografts (Southam, 1968; Green & Corso, 1959; Miller, Lizardo & Snyderman, 1961; Levin *et al.*, 1964a) and diminished delayed hypersentivity responses (Levin *et al.*, 1964b; Aisenberg, 1966), in spite of normal serum antibody production, indicates that either circulating antibody is not involved in delayed type responses or that it is qualitatively defective. Although there is considerable evidence favouring the former explanation (Good *et al.*, 1959; Itoh & Southam, 1964; Tanigaki & Southam, 1966) the latter cannot be dismissed, so further knowledge of the quality of antibody response in cancer patients is needed, particularly in view of recent advances in knowledge of the structure and immunologic function of the serum globulins (Fahey, 1965).

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This is a report of antibody responses in healthy persons and patients with neoplastic diseases, with differentiation of the antibody into two categories: '7S antibody' as defined by persistence of activity after incubation with mercaptoethanol, and rapid elution from a DEAE cellulose column by low ionic strength buffer at pH 7·4; and '19S antibody' as defined by the opposite characteristics.

MATERIALS AND METHODS

Vaccine

The stimulus for antibody production was the standard 17D strain yellow fever vaccine U.S.P. (National Drug Co., Philadephia, Pa., U.S.A.) which is an attenuated live virus preparation. A single dose of 0·5 ml of the vaccine was injected subcutaneously immediately after reconstitution, as prescribed for clinical practice.

Subjects

Thirty-nine persons, from whom serial specimens of sera were obtained at approximately weekly intervals for at least 3 weeks after vaccination and who had no detectable antibodies to yellow fever prior to the vaccinations are included in the antibody studies. Eleven of these were healthy controls (six men, five women, mean age 35 years, range 20–58); twenty-four had epidermoid carcinoma or adenocarcinoma and are referred to hereafter as the carcinoma group (fourteen men and ten women, mean age 57 years, range 34–75). The other four had lymphomatous neoplasms (three men and one woman, mean age 43 years, range 38–51). Of the carcinoma patients, eleven had epidermoid carcinoma, (ten head and neck cancers and one lung) and thirteen had adenocarcinoma (eight breast, two lung, one stomach, one colon and one thyroid). Two of the lymphoma group had Hodgkin's disease, one had lymphocytic leukaemia and the other had myeloma. All patients had distant metastases or advanced regional extension of their cancers. Many were debilitated and some were bedridden. Eleven of the twenty-four carcinoma patients and all four of the lymphoma patients received antineoplastic therapy which might have been immunosuppressive.

Antibody titration

Antibody was titred by the haemagglutination inhibition technique (HAI) of Clarke & Cassals (1958) in which the amount of antibody is expressed as the greatest dilution of serum which will prevent agglutination of chick erythrocytes by yellow fever virus under standardized conditions. The virus haemagglutinin was prepared from the brains of suckling mice infected with the 17D strain of yellow fever virus. Sera were acetone extracted and adsorbed with packed chick erythrocytes to remove non-specific haemagglutinins. All specimens from each individual were tested simultaneously and each run included the sera from at least one cancer patient and one healthy control.

A few selected sera were studied for virus neutralizing antibody, using both virus dilution and serum dilution techniques in tissue cultures of the human cell line RPMI-41 (Moore & Koike, 1964).

Fractionation of serum globulins

The principal technique used to distinguish 19S and 7S antibody was based on the selective destruction of the former by 2-mercaptoethanol (MCE), (Uhr & Finkelstein, 1963). This was

checked by fractionating a few of these same serum specimens on DEAE cellulose columns (Fahey, 1962), measuring protein concentration by absorption of ultraviolet light at 280 mμ, and titrating the fractions for HAI antibody.

Viraemia and interferon

Additional sera from thirty-two persons were collected on alternate days during the first week following yellow fever inoculation, and tested for infectious virus by intracerebral inoculation into newborn mice. Many were also tested for interferon using the technique of Wheelock & Sibley (1965).

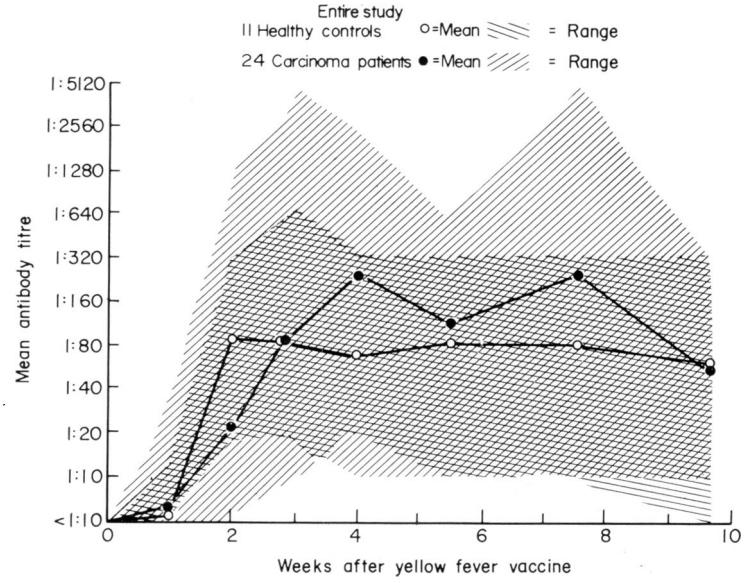

FIG. 1. Geometric mean titres and range of HAI antibody after 17D yellow fever vaccine in whole serum of carcinoma patients and healthy individuals.

RESULTS

Total HAI antibody response

In most persons antibody was not detectable at 1 week post inoculation (Fig. 1). By 2 weeks all eleven healthy controls and fifteen of the twenty-one carcinoma patients who were tested, had detectable antibody (1:10 or greater). At 3 weeks all carcinoma patients had titres of 1:10 or greater, and the mean titres of these patients and the healthy controls were identical (1:160). After the third week, mean titres were slightly but persistently higher in the carcinoma group than in the healthy controls. Individual values varied considerably from the means, but the range was similar in all groups.

The slight difference in mean antibody titres at 14 days persisted even after excluding the six patients who had no detectable antibody at 2 weeks. Of these six, four received anti-cancer therapy which might have impaired the immune response (prednisone, extensive

radiotherapy to the trunk, mustargen), but the other two had no such treatment and were not lymphopenic, so therapy does not appear to be the cause of the delayed antibody response. Furthermore, four patients who received radiotherapy or chemotherapy during the immediate pre- and post-immunization period did produce antibody by 14 days at titres from 1:40 to 1:1280. Of the six carcinoma patients who did not have demonstrable antibody at 2 weeks, two died before a 3rd week serum was obtained, but the other four did have antibody at 3 weeks at titres within the control range. These four were also close to death (died on days 24, 40, 75 and 90), but the terminal state does not account for their slow antibody response, since three patients who died on days 17, 29 and 40 had antibody titres of 1:1280, 1:40 and 1:10 respectively on day 14, and the latter two reached titres of 1:1280 and 1:80 by 3 weeks.

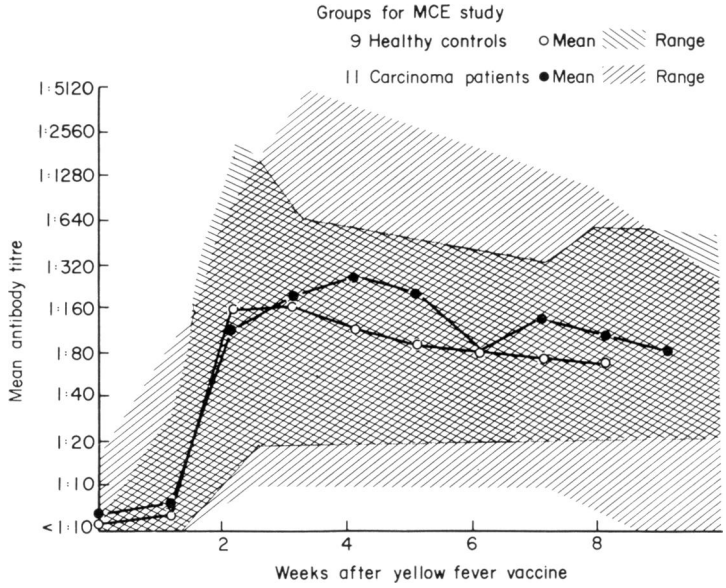

FIG. 2. Geometric mean titres and range of HAI antibody in whole serum of those carcinoma patients and healthy individuals whose antibodies were studied for mercaptoethanol sensitivity.

The four lymphoma patients are not included in Fig. 1. At 1 and 2 weeks none of them had detectable antibodies, but by 3 weeks all had titres within the range of the healthy controls. All four lymphoma patients were receiving anti-tumour drugs so it is not known whether the slightly slower antibody response was an effect of the disease or the treatment.

Mercaptoethanol fractionation of HAI antibody

Fig. 2 shows total antibody levels in those carcinoma patients and controls whose antibodies were also studied for MCE sensitivity. They were selected to include both higher and lower ranges of antibody production, and each had a complete or nearly complete series of weekly serum samples through week 6 or later. Of the cancer patients, six had epidermoid carcinomas of the head and neck region, four had mammary adenocarcinoma, and one had gastric carcinoma.

Mean titres of mercaptoethanol resistant (7S) antibody in these same persons are shown in Fig. 3. Maximum titres were not reached until the 4th week because much of the antibody in earlier specimens was 19S. At 1 and 2 weeks, mean titres of 7S antibody were low, and almost identical in patients and controls. Thereafter, they plateaued in the healthy controls, but continued to rise in the carcinoma group to reach maximum at 4 and 5 weeks—as was also seen in the total antibody titres.

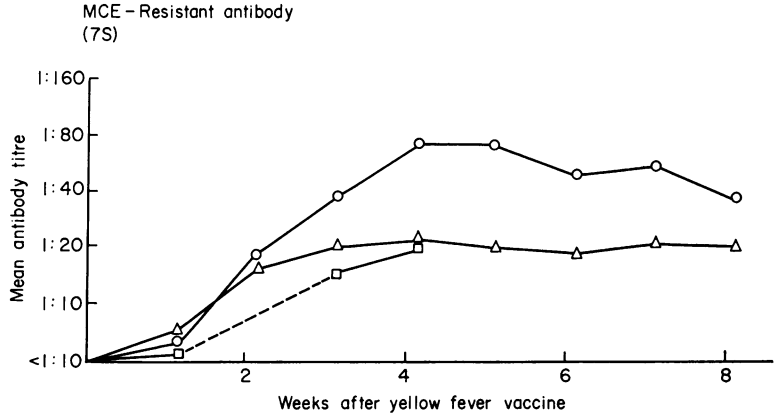

FIG. 3. Geometric mean titres of 7S (mercaptoethanol resistant) antibody in healthy controls, carcinoma patients and lymphoma patients. The only 2-week serum specimen from a lymphoma patient showed no HAI antibody and is omitted from this graph and from Fig. 4. △, nine healthy controls; ○, eleven carcinoma patients; □, three lymphoma patients.

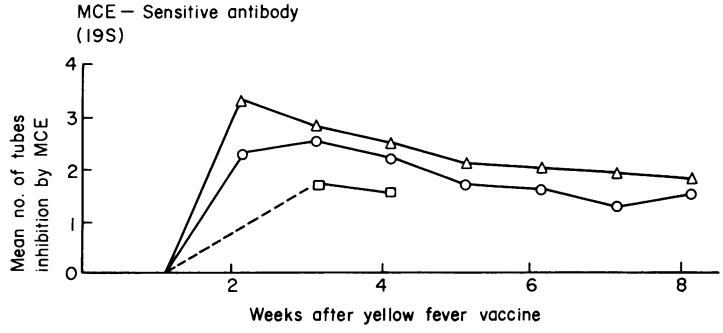

FIG. 4. Average levels of 19S (mercaptoethanol sensitive) antibody in healthy controls, carcinoma patients and lymphoma patients. Antibody levels are expressed as the number of tubes (2-fold serum dilutions) by which the antibody titre of whole serum was diminished after incubation with mercaptoethanol. Details of symbols as Fig. 4.

Average levels of 19S antibody are shown in Fig. 4. Because the 19S antibody was measured as the loss of activity after treatment of sera with MCE, these data are recorded as the number of tubes (two-fold serum dilutions) by which antibody titre was decreased by the MCE treatment. No 19S antibody is shown at 1 week because the mean titre of total serum

antibody was less than 1:10 and consequently the level of 19S antibody was less than one tube. Nevertheless, the low levels which were present in some day 7 sera were completely inactivated by MCE, indicating that all or most of the first antibody to be produced was 19S globulin. At 2 and 3 weeks 19S antibody still predominated. By 4 weeks it fell in both controls and carcinoma patients. Sera from three of the lymphoma patients (one Hodgkin's disease, one myeloma, one lymphocytic leukaemia) were tested for MCE sensitivity. At 3 and 4 weeks 19S antibody predominated but was slightly less than in the carcinoma patients and controls, while 7S levels were virtually identical in all groups. The only later serum was from the Hodgkin's disease patient at 6 weeks, and was all 7S.

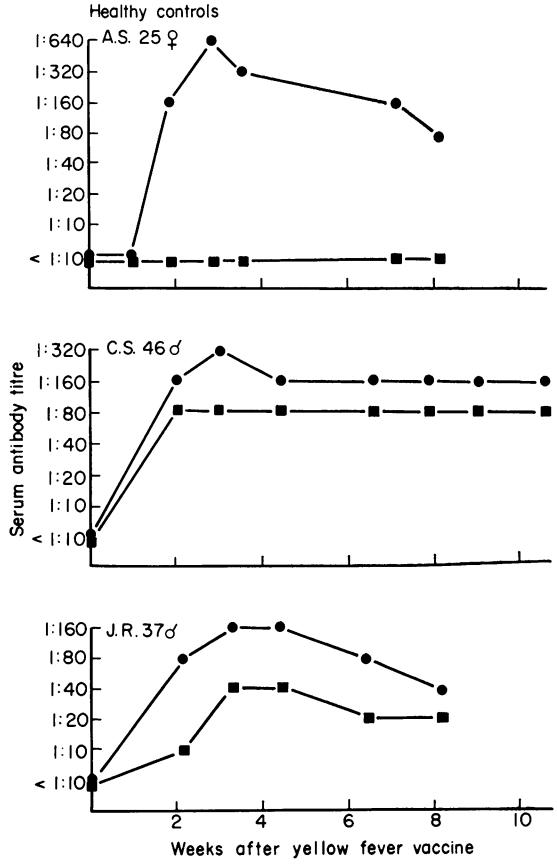

FIG. 5. HAI antibody in whole serum and mercaptoethanol-treated serum of three healthy controls showing various patterns of 7S and 19S antibody response. ●, Total antibody; ■, MCE-Resistant Antibody (7S).

Among individual patients and controls there was considerable variety in magnitude and type of antibody response. In healthy controls (Fig. 5) three patterns were distinguished: (1) prompt and continuous production of 19S antibody only for the duration of the study (subject A.S.); (2) production of 7S antibody early and predominantly (subject C.S.); and (3) prompt decrease in 19S antibody levels with a preponderance of 7S antibody by 3 or 4

weeks. The latter pattern was most common and dominated the averaged data, but all three are presumably variants of the normal response.

These three patterns of response were also observed among the carcinoma patients (Fig. 6). Patient MS had 'pure' 19S antibody at $3\frac{1}{2}$ and $5\frac{1}{2}$ weeks, but had no detectable antibody at $8\frac{1}{2}$ weeks. Patient JM had a prompt 7S antibody response with minimal 19S antibody on day 7 only. Patient AH had predominantly 19S antibody at $3\frac{1}{2}$ weeks, gradually replaced by 7S thereafter. This was the most common pattern in the carcinoma patients, as in the healthy controls.

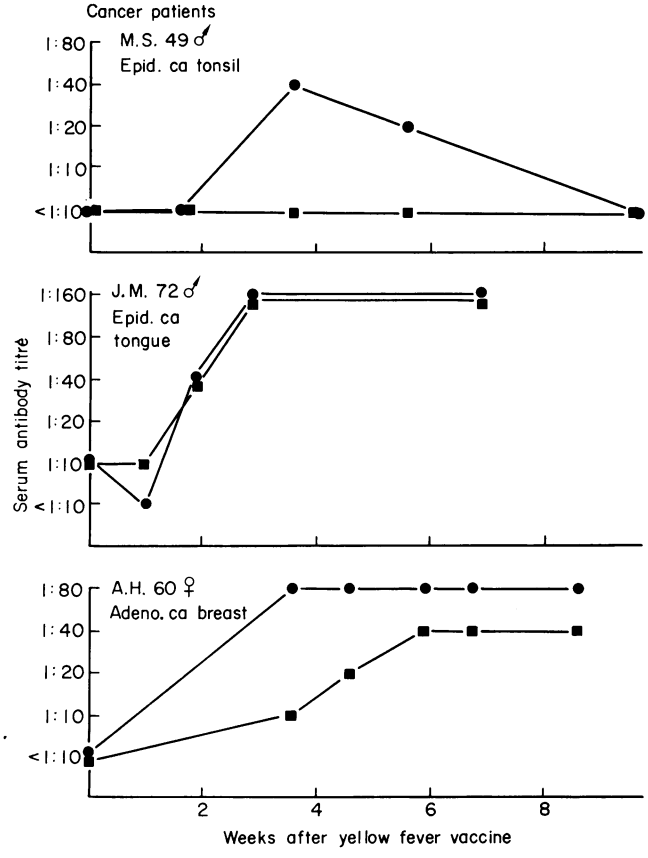

FIG. 6. HAI antibody in whole serum and mercaptoethanol-treated serum of three carcinoma patients showing various patterns of 7S and 19S antibody response. ●, Total antibody; ■, MCE-resistant antibody (7S).

DEAE fractionation of HAI antibody

Five sera with varied reactions to MCE were fractionated on DEAE cellulose to check validity of the MCE technique for distinguishing 19S from 7S antibodies. Results of the two methods were in agreement for all five sera. Figs 7, 8 and 9 illustrate, respectively, the data from one serum which contained both 7S and 19S antibody, one which showed only macroglobulin antibody, and another which had only 7S antibody.

Neutralizing antibody response

A day 23 serum from a healthy subject and a day 24 serum from a carcinoma patient were tested by the serum dilution technique with serial two-fold dilutions of serum and fifty tissue culture LD_{50} doses of virus. One serum titred 1:160 by both techniques and the other was 1:1280 by neutralization and 1:5120 by HAI. No MCE-treated sera were studied by this method.

Eighteen sera from four healthy controls and eight carcinoma patients were tested by the virus dilution method with undiluted serum. Four pre-immunization sera did not inactivate virus. The other fourteen sera all neutralized two to three logs of virus (that is, reduced

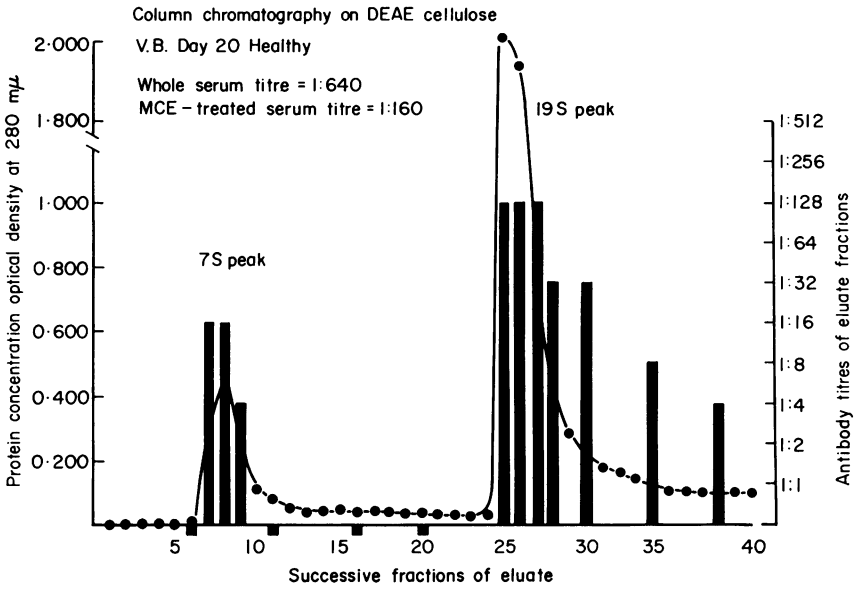

FIG. 7. Chromatography study of a day 20 serum from a healthy control. The horizontal axis indicates successive 10 ml fractions of eluate. The curve shows relative protein concentrations in these fractions as measured by U.V. absorption at 280 mμ. The higher molarity buffer was put on the column after fraction 15, so 7S globulins are in the first peak and 19S globulins first come through about fraction 25. Vertical bars show relative HAI antibody content of the same fractions, expressed as the greatest dilution of the eluate fraction which showed antibody activity. The short black bars below the horizontal axis indicate no detectable antibody. This serum contained both 7S and 19S antibody.

infectivity by 99·0–99·9%) while HAI titres ranged from 1:10 to 1:2560. There was no correlation between HAI titre and amount of virus neutralized. Four of these sera were tested for neutralizing antibody after incubation with mercaptoethanol, and dialysis to remove the excess MCE. In two, all neutralizing antibody was 19S, another was partially inactivated, and the fourth was all 7S. The same results were obtained with these four sera in the HAI antibody titrations, indicating that haemagglutination inhibition and neutralization are properties of both 7S and 19S antibodies.

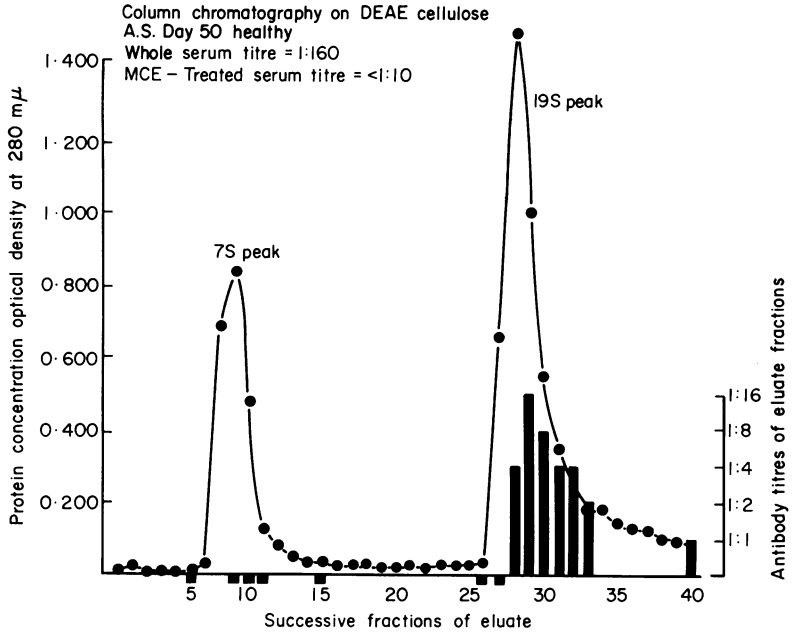

FIG. 8. Chromatography study of a day 50 serum from another healthy control. Only 19S antibody was found in this serum.

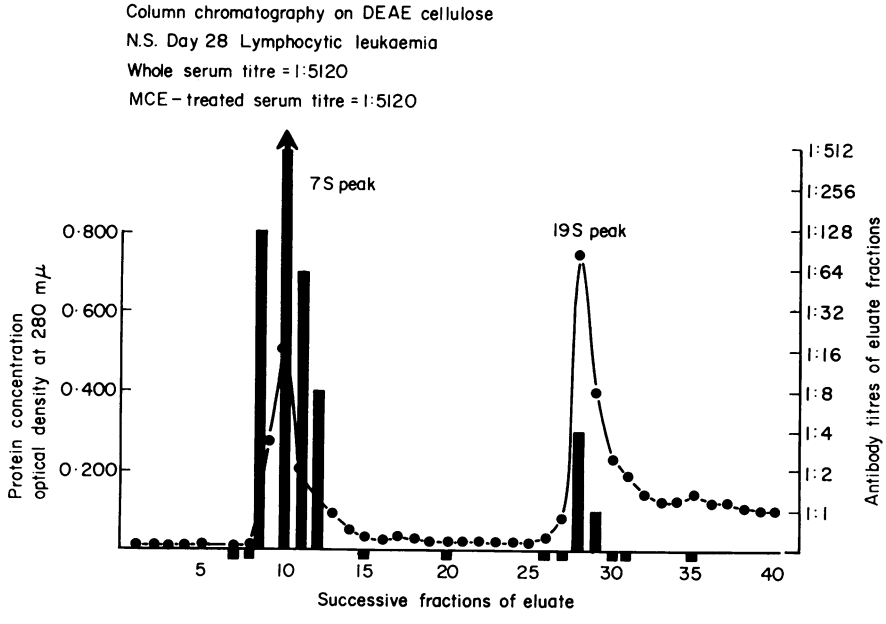

FIG. 9. Chromatography study of a day 28 serum from a patient with chronic lymphatic leukaemia. Most of the antibody was 7S.

Viraemia Studies

Nineteen blood specimens collected from eight healthy persons during the 1st week after virus inoculation were tested for infective virus. Seven persons had no demonstrable viraemia. The other had yellow fever virus in his blood on days 5 and 7 but not on day 3. He had a prompt antibody response (c.s. Fig. 5).

In the lymphoma group, the leukaemia patient had no detectable viraemia. One of the Hodgkin's disease patients had viraemia on day 5, but not on day 3, and the other had viraemia on day 6, but not on day 1 or 3.

Fifty-four sera were collected during the week after virus inoculation from twenty carcinoma patients (eleven head and neck cancers, seven breast, one colon, and one kidney). Four patients (all breast cancer) had viraemia: two on day 5 but not on days 3 or 7: one on day 7 but not on days 3 or 5; the fourth on days 1 and 6 but not on day 4. Each had HAI antibody by 14 to 21 days at levels of 1:160 to 1:5120. None of the viraemic subjects had signs or symptoms attributable to the virus.

Interferon studies

Fifty-four sera collected from day 1 to day 7 from forty-two persons (nine healthy, six lymphomas, twenty-seven carcinomas) were adequately tested for interferon. Only three caused any delay in viral cytopathology (day 2 and 4 sera from a healthy control and day 6 serum from a breast cancer patient).

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