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(54) PROCESS FOR THE PREPARATION OF AN ANTISERUM SPECIFIC TO HUMAN CARCINOEMBRYONIC ANTIGEN

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Abstract

A process for the preparation of antiserum specific for carcinoembryonic antigen is disclosed as well as the antiserum so produced.

The present invention is concerned with a process for the preparation of antiserum specific for carcinoembryonic antigen as well as the antiserum so produced.

The present application is divided from Application serial No. 114,464 which describes and claims a method for detecting the presence of carcinoembryonic antigen in blood which comprises (a) adding a measured amount of anti-CEA antiserum to a sample of perchloric acid extracted blood serum; (b) incubating the mixture; (c) adding a measured amount of radioactive tagged carcinoembryonic antigen to the incubated mixture; (d) incubating the mixture; (e) adding a protein precipitant to the incubated mixture thereby coprecipitating all the CEA-anti-CEA-complexes; and (f) measuring the radioactive content of the supernatant or precipitate.

The antiserum of the present invention is useful in the diagnostic test of said Application said No. 114,464. Reference should also be made to our copending Application serial No. (RAN 4060/46-001) which describes and claims human carcinoembryonic antigen and also the radioiodinated form of human carcinoembryonic antigen.

According to the present invention there is provided a process for the preparation of anti-CEA antiserum specific to human CEA, which comprises treating a serum, from animals immunized with processed adenocarcinoma tissue, originating in the digestive system epithelium derived from embryonic entodermal tissue, with normal colon tissue extract.

The treatment with normal colon tissue extracts serves to remove other antibodies by absorption.

This invention thus involves immunizing animals with purified CEA. An emulsifier, e.g. Freund's adjuvant (complete) is added to CEA in a saline solution. The emulsion can be injected in animals intramuscularly, subcutaneously, in the foot pad or any combination of these methods. Animals such as fowl, rabbits, horses, goats, sheep and the like are suitable. The regimen in rabbits, for example, is injections twice a week until five injections are made. After the last injection, blood is collected from the animal.The serum from this blood is (anti-CEA)-antiserum and contains the antibody in unabsorbed form.

In one method, 400 µg of CEA in 1 ml of saline solution (0.9%) is utilized. The injection is made intramuscularly using a volume about four times that injected in the foot pad.

The antibody present in the antiserum, after removal of other antibodies by absorption with normal tissue components, is specific in its activity against CEA to the exclusion of other antigens.

The invention will now be illustrated with reference to the following Example.

Example

(A) Preparation of anti-CEA antiserum:

Twelve adult, male, New Zealand white rabbits weighing 2.0 kg were divided into 3 groups of 4 each. The different groups were immunized with the following materials: a) normal colon tissue extract, b) tumour tissue extract from the same individual from whom the normal tissue extract was obtained and c) pooled human plasma. Groups a) and c) were control groups. The tumour tissue extract was from specimens of tumours diagnosed and confirmed to be adenocarcinoma of the colon. The normal tissue extracts and tumour tissue extracts were formed in accordance with the procedure described in part (A) of the Example of our copending Application serial No. 114,464. The pooled human plasma was obtained from 30 normal donors representing all major blood groups.

The injections which were given twice weekly for 4 weeks contained 3 mg of protein in 0.6 ml of tissue extract or plasma emulsified in an equal volume of Freund's adjuvant (complete). Injections of 0.1 ml to 0.2 ml were given into a foot-pad and the remainder intramuscularly into the flank. Twelve days following the last injections, the animals were bled from their marginal ear veins and the sera obtained from each group was pooled separately for testing.

The sera from each group was absorbed with normal tissue and the resulting precipitate separated. The resulting antiserum of group b) was suitable for use in the diagnostic test described and claimed in our copending Serial Application No. 114,464.

In order to confirm that the serum contained antibodies specific for the tumour, the supernatant liquids were subjected to the Ouchterlony technique of double diffusion in agar gel. This was performed in one per cent agar-in-saline with merthiolate added as a preservative to a final concentration of 1/10,000. The patterns were cut in the gel plates so that the central and peripheral wells were spaced 1.0 cm apart. Each well was filed with 0.15 ml of test material. The antigen concentration used was 10 mg protein per ml. Initial incubation of the plates was carried out in a moist environment at 37°C for 24 hours to encourage diffusion of material from the wells. Patterns were allowed to develop in the same humid atmosphere at 25°C for seven days. The patterns from the serum of the animals which were immunized with tumour tissue extract [group b)] were a single line, indicating the serum contained antibodies specific for the tumour. No patterns developed from the sera of animals immunized with normal tissue or normal human plasma.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. In a process for the preparation of anti-CEA antiserum specific to human carcinoembryonic antigen, the step which comprises treating a serum, from animals immunized with processed adenocarcinoma tissue, originating in the digestive system epithelium derived from embryonic entodermal tissue, with normal colon tissue extract.

2. Antibodies specific for human carcinoembyronic antigen whenever prepared by a process as claimed in claim 1.

SUBSTITUTE

REMPLACEMENT

SECTION is not Present

Cette Section est Absente