of "tumor take," which would result in larger final tumor weight.

Since Miller, Fancher and Bale(9) reported that complete removal of liver would inhibit incorporation of lysine-C^{14} into Walker tumor, and in view of our studies it seemed a small amount of liver tissue could provide the necessary nutrients for neoplasm even during period of rapid tumor growth. Under these conditions, partial hepatectomy did not affect final tumor weight but did enhance carcass weight gains.

Summary. Liver weights of rats bearing 40-60 g Walker carcinosarcoma 256 tumors were directly correlated with changes in carcass weight of host. Removal of 70% of liver during rapid growth of tumor resulted in a gain in carcass weight without affecting growth of the tumor.

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Antigenicity of Connective Tissue Extracts. (24999)

PAUL HELLER, VINCENT J. YAKULIS AND H. J. ZIMMERMAN

Research Lab. and Medical Service, VA West Side Hospital and Depts. of Medicine, University of Illinois College of Medicine and Chicago Medical School

The immunologic concept of pathogenesis of "collagen diseases" has stimulated interest in studies of antigenicity of connective tissue and its components. Such investigations resulted only rarely in successful production of specific antibodies. Watson, Rothbard and Vanamee(1) induced complement fixing antibodies to acetic acid extracts of rat collagen and concluded that these antibodies were specific to collagen. Attempts to demonstrate antigenicity of hyaluronic acid(2) and chondroitin sulphate(3) have not been successful. In previous experiments, reported in abstract only(4), injection of saline extracts from rabbit tendons into guinea pigs did not elicit anticonnective tissue antibodies, but in serum of injected guinea pigs high levels of complement fixing antibodies to saline extracts of rabbit and human leukocytes were demonstrated. This apparent experimental immunologic relationship of connective tissue and leukocyte extracts was of great interest in view of immunologic abnormalities in acute disseminated lupus erythematosus. When other batches of tendon extracts failed to stimulate generation of such antileukocytic antibodies a search was made for the cause of this variability in antigenic behavior of saline extracts from rabbit tendons. Results of the present study indicate that neutral salt extracts from leg tendons of aged rabbits, combined with Freund's adjuvant, elicit a greater antibody response in guinea pigs than such extracts from tendons of young animals. In contrast to previous experiments anticonnective tissue antibodies were demonstrable. These anticonnective tissue sera also crossreacted with the cytoplasmic fraction of rabbit leukocytes.

Methods. Extracts of connective tissue were prepared as follows: Tendons from small muscles of lower extremities of 7-day-old and aged rabbits (older than 1 year) were carefully separated from muscle tissue, washed in running tap water, soaked in cold tap water over-

night and transferred into physiologic saline solution* (10 ml/g wet tissue). They were then ground 10 minutes in cooled Waring blendor. To achieve a final protein concentration of 150 μg/ml (determined according to method of Lowry(5)) the filtrate was either diluted with saline or concentrated by dialysis against a 30% solution of Polyvinylpyrrolidone. This material was then either used fresh or stored at −20°C for later use. The filtrate was mixed with equal amount of Freund's adjuvant. Aliquots of 1 ml of this mixture were injected subcutaneously or intramuscularly into multiple sites of male guinea pigs of Hartley Strain, weighing approximately 250 g. These injections were given at weekly intervals for total of 4 ml (300 μg of protein). Blood was obtained by cardiac puncture 10 days after final injection. Thirty-one guinea pigs were injected with connective tissue extracts obtained from old animals (subsequently called "O.C.T.") and 32 animals were injected with such extracts obtained from young animals (subsequently called "Y.C.T."). Three animals were injected with Freund's adjuvant alone and 5 with rabbit serum. Leukocytic extracts were prepared according to method of Miescher(6) which permits separation into cytoplasmic and nuclear fraction. The cytoplasmic fraction was then dialyzed against distilled water to remove sucrose and citric acid from original extract and adjusted to isotonicity by addition of sodium chloride and further diluted, if necessary, with physiologic saline to result in protein concentration of 150 μg/ml. The nuclear fraction, soluble in 1 M saline, was adjusted to concentration of 150 μg/ml by addition of distilled water until isotonicity was achieved. If further dilution was necessary for desired concentration, saline was used. In the process of dilution the nuclear material precipitated, but could be maintained in suspension. Two groups of 5 animals each were injected with these fractions prepared from rabbit leukocytes. All substances with exception of adjuvant were also used as test antigens in the complement fixation test, performed according to standard method of Kolmer. For demonstration of precipitins the agar double diffusion technic of Ouchterlony as modified in this laboratory(7) was used. Statistical analysis was performed by chi-square method following grouping of results into 4-fold contingency tables.

* Saline solution used in immunology laboratory contains 0.85% sodium chloride, 0.01% magnesium sulphate, and 0.004% calcium chloride.

TABLE I. Complement Fixing Antibodies to "Y.C.T." and "O.C.T."

Titer*	No. of animals sensitized with "Y.C.T."	"O.C.T."
<8	21	9
>8	11	22

* "O.C.T." used as test antigen; "Y.C.T." ineffective as test antigen (Fig. 1).

$X^2 = 7.3$; $P < .01$.

Results. The majority of guinea pigs injected with "Y.C.T." developed complement fixing antibodies of low titer (Fig. 1, Table I) with the use of "O.C.T." as test antigen. With "Y.C.T." as test antigen the titers were even smaller, only 3 out of 32 guinea pigs had antibody levels at 8 and 16 fold dilution. Thus, "Y.C.T." was not only a poor sensitizing but also a poor test antigen.

In contrast, 22 out of 31 guinea pigs injected with "O.C.T." had antibody levels demonstrable at higher than 16 fold dilution (Fig. 1). The difference in antigenicity between "O.C.T." and "Y.C.T." was statistically significant at the 1% level (Table I).

No complement fixing antibodies to connective tissue extracts could be found in animals injected with Freund's adjuvant alone or with

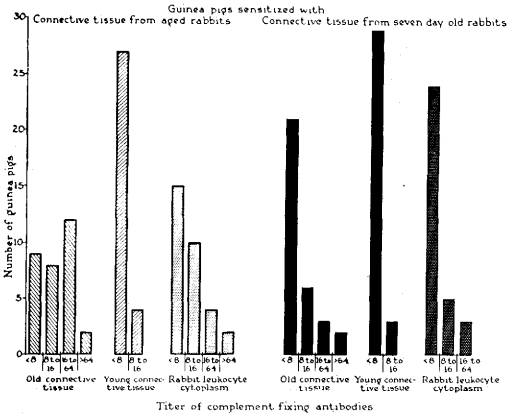

FIG. 1. Levels of antibodies to "O.C.T." and "Y.C.T." (see text for explanation).

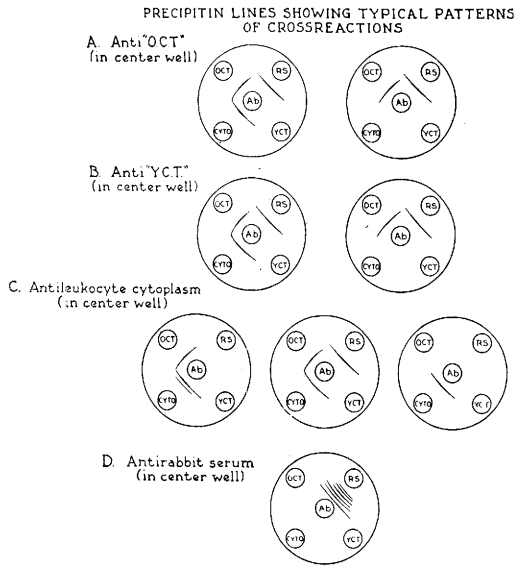

FIG. 2. Precipitin lines in double diffusion agar. Ab—Antirabbit serum, OCT—"Old" connective tissue, YCT—"Young" connective tissue, CYTO—Leukocyte cytoplasmic extract, RS—Native rabbit serum.

serum. No precipitins were demonstrable in the test tube on overnight incubation, but with agar double diffusion technic precipitin lines were present between "O.C.T." as test antigen and antisera to "O.C.T." (Fig. 2A). Such precipitins were also seen between "O.C.T." as test antigen and antisera to "Y.C.T.", but no precipitins occurred when "Y.C.T." was used as test antigen. As with the complement fixing test "Y.C.T." was also a poor test antigen for the demonstration of precipitating antibodies.

A precipitin reaction was also noted between antisera to "O.C.T." and native rabbit serum (Fig. 2A). On the other hand no such reaction was seen between guinea pig serum containing anti-rabbit serum antibodies and "O.C.T." or "Y.C.T." (Fig. 2D).

Animals injected with "O.C.T." and "Y.C.T." developed complement fixing and precipitating antibodies to leukocyte cytoplasm (Figs. 1, 2). In the complement fixation test higher antibody levels were observed in guinea pigs sensitized with "O.C.T.", but the difference between these animals and those injected with "Y.C.T." was not statistically significant ($0.1>P>0.05$, Table II). No complement fixing antibodies to nuclear antigen could be demonstrated in these guinea pigs.

TABLE II. Production of C.F. Antibodies to Leukocytic Cytoplasm with "Y.C.T." and "O.C.T."

Titer	No. of animals sensitized with "Y.C.T."	"O.C.T."
<8	24	15
>8	8	16

$X^2 = 3.8$; $P > .05$ (for $P = .05$, X^2 of 3.84 would be required).

In sera of animals injected with leukocyte fractions, complement fixing antibodies to the respective fractions were demonstrated, with slight crossreaction between cytoplasmic and nuclear material. In the Ouchterlony preparation, however, crossreaction of anticytoplasmic antibodies with "O.C.T." was apparent. They also reacted with rabbit serum (Fig. 2C).

Guinea pigs injected with connective tissue extracts failed to gain weight and their fur became sparse (Fig. 3). Pathological findings in these animals are being investigated. There was apparently no difference in this reaction whether animals were injected with "O.C.T." or "Y.C.T.". No animals injected with the other substances exhibited these alterations.

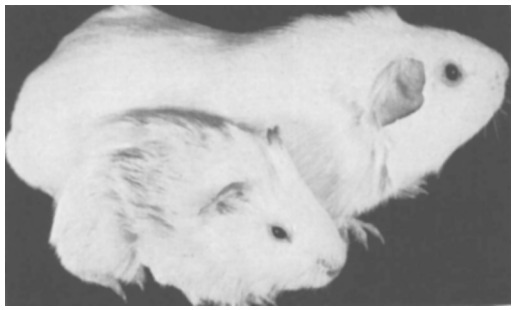

FIG. 3. Appearance of guinea pigs 7 wk after start of experiment. Rear—Control animal. Front—"O.C.T." inj. guinea pig.

Studies on chemical characterization of the antigenic component of connective tissue extracts are in progress. Preliminary observations indicate presence of hydroxyproline in hydrolysates of these connective tissue extracts.

Discussion. Tendons consist predominantly of 25-30% collagen and 0.75-1.5% of mucoproteins(8). The bulk of the remainder is

water. According to Meyer(9) mucopolysaccharides of the ground substance of tendons consist predominantly of chondroitin sulphate B and C which are firmly bound to protein. Chondroitin sulphate B cannot be extracted with water or neutral salt solutions which were used in our experiments. Chondroitin sulphate C is slightly more soluble in such neutral salt solutions. Collagen is not only the protein of fibrils, but a fraction of it appears dispersed in the ground substance (10) and can be extracted with neutral salt solutions of low molarity. This neutral salt soluble fraction of collagen is small, except in actively growing tissue(11) and in connective tissue stimulated into active growth by carrageenin(12). All connective tissue extracts used contained the same amount of protein. Nevertheless, there was a marked difference in antigenicity between connective tissue extracts from old and those from young rabbits. The cause of this is still obscure. It is possible that because of lower solubility of "adult" collagen in neutral salt solutions a larger amount of it had to be extracted to yield 150 μg of protein/ml than from "young" connective tissue. This could have resulted in a higher concentration of substances which might be responsible for the greater antigenicity. It is not likely that these substances are mucopolysaccharides as it has been shown (13) that concentration of sulphated polysaccharides in neutral salt solutions of connective tissue is directly proportional to concentration of neutral salt soluble fraction of collagen.

The difference in antigenicity between "O.C.T." and "Y.C.T." was demonstrated mainly in the complement fixation test. On the other hand "young" connective tissue stimulated development of precipitins in sera of injected guinea pigs as well as "O.C.T.", but these precipitins could only be demonstrated with "O.C.T." as test antigen.

In the precipitin test antisera to connective tissue preparations also reacted with serum. Could this crossreaction with serum be interpreted as serum contamination of connective tissue extracts? This is not likely. Native tendons contain only a small amount of plasmaproteins(8), and moreover it is likely that this amount was further reduced by preparation of the extracts. The fact that anti-rabbit-serum antibodies did not react with connective tissue extracts, is not consistent with the possibility of presence of rabbit serum in these extracts. It is more likely that the rabbit serum contained a small amount of antigenic substance of connective tissue extracts.

The crossreaction of anticonnective tissue antibodies with rabbit leukocyte cytoplasm is of great interest. In previous experiments antileukocytic antibodies were demonstrated in guinea pigs sensitized with connective tissue extracts(4). As test antigen saline extracts of rabbit and human leukocytes were used. No attempt was made to separate leukocytes into cytoplasmic and nuclear fractions. In our experiments it could be shown that only cytoplasmic fraction of leukocytes reacted with anticonnective tissue sera. The failure to demonstrate antigenicity of nuclear extracts in the precipitin test seems relatable to their insolubility in physiologic saline and to resulting incapacity to diffuse in agar gel. In the complement fixation test, however, this technical problem does not exist, as evidenced by the demonstration of homologous antinuclear complement fixing antibodies. The fact, therefore, that in this test anticonnective tissue sera did not react with nuclear extracts, speaks in favor of a lack of an immunologic relationship between extracts of leukocyte nuclei and connective tissue.

Preliminary observations indicate presence of hydroxyproline in hydrolysates of cytoplasmic extracts of rabbit leukocytes. This could permit the conclusion that these leukocytes contain collagen. It remains to be proved whether this explains the apparent immunologic relationship between connective tissue and leukocyte cytoplasm.

Summary. 1. Antibodies to rabbit connective tissue have been induced in the guinea pig. 2. Connective tissue extracts from aged rabbits had greater antigenicity than from young animals. 3. Anticonnective tissue serum crossreacted with the cytoplasmic fraction of rabbit leukocytes. 4. Chemical characteristics of the antigenic component of connective tissue remain incompletely explored.

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Disease in Macacus Monkeys Inoculated with ECHO Viruses.* (25000)

JOHN H. ARNOLD† AND JOHN F. ENDERS

Research Division of Infectious Diseases, Children's Medical Center, Boston

Several ECHO viruses have been implicated as causative agents of neurologic disease in man(1). Paralysis has occurred in individual patients infected with some of these viruses(2,3,4,5). The recent reports of Steigman(6), Dalldorf(7) and of Habel and Loomis(8) leave no doubt that paralysis and neuronal damage can be induced in monkeys experimentally infected with Coxsackie viruses representative of both A and B groups. These investigators observed microscopic changes in the central nervous system of inoculated animals which were indistinguishable from those caused by poliovirus. Wenner and Chin(9) in monkeys used for preparation of antiserum against ECHO prototype strains described degenerative changes in neurons after the animals had received several injections of virus and mineral oil adjuvant. Our experiments were undertaken to determine whether 2 types of ECHO virus could produce disease of the central nervous system in monkeys.

Materials and methods. Tissue cultures. Cultures were prepared according to previously described technics(10,11). Cultures of human amnion cells were used in attempts to demonstrate virus in materials derived from inoculated monkeys and in neutralization tests with their serum. The *viruses* employed were ECHO Type 6, strain SHO(2) and ECHO Type 16, strain HAR(2). Clinical manifestations in patients infected with these 2 strains have been described by Kibrick *et al.*(2). The strains were recovered in tissue culture from fecal specimens. The type 6 strain was isolated in human kidney cells and subsequently passed once in this medium and twice in human amnion cells. The fluid from the last of these passages had an infectivity titer of $10^{-6.5}$/0.1 ml in amnion cells and was used as inoculum in the experiments to be described. The type 16 strain was recovered in human kidney cells, passed twice in human embryonic skin and muscle tissue, and twice in human amnion cells. The fluid from the last of these passages had an infectivity titer of $10^{-3.5}$/0.1 ml in cultures of human amnion cells, and was used as inoculum. *Monkeys.* Seven rhesus and 3 cynomolgus monkeys were studied. Before each was injected, specimens of blood for antibody determination, heparinized blood for viral culture, and peripheral blood for white blood cell counts were obtained. Then a #22 gauge hypodermic needle was introduced into the basal cistern, and about 1-2 ml of cerebrospinal fluid was withdrawn for white cell count and for viral culture. Eight of the monkeys were inoculated with 0.5 ml of undiluted tissue culture fluid containing the virus which was introduced into the basal cistern. In half of the animals

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† Supported by fellowship from Commonwealth Fund. Present address: Univ. of North Carolina School of Med., Chapel Hill.