of ROITT et al.[3], who have established that the cytoplasmic complement fixing antigen of the human thyroid epithelium is soluble either in ethanol or in methanol. The presence of LATS in the serum in no manner affected this negative fluorescent reaction. This shows the specificity of the technique of HOLBOROW et al.[6] for the detection of the complement fixing antibody, and its unfitness for the detection of LATS in the serum.

The discrepancies between the results of BLUM et al.[1] and the data presented here may be due to a different reactivity of the guinea-pig thyroid gland in the fluorescent assay. Up to now, however, no such different behaviour has been proved.

Résumé. La technique immunofluorescente de COONS, sur coupes de thyroïdes thyrotoxiques humaines, fixées ou non fixées, a été appliquée à des sérums de sujets hyperthyroïdiens et de sujets atteints de thyroïdite atrophique asymptomatique. Cette technique détecte spécifiquement l'anticorps anticytoplasmique fixant le complément. Elle ne permet pas la mise en évidence du LATS.

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Specific Anti-Antibody in Transplantation

In recent years, the concept that antibody globulins show no immunologically recognizable differences from normal globulins has been refuted. Various investigators have demonstrated that anti-antibodies can be produced[1–5], although the exact mechanism of their action is unknown. It has been suggested, however, that anti-antibody may be directed against the binding site of the antibody used as antigen[3].

To investigate the potential use of such anti-antibodies in a therapeutic sense, we propose that it may be possible to train the individuals' lymphopoietic system to produce anti-antibodies against certain antibodies produced by the recipient in homograft rejection, or against antibodies present in certain immune diseases. To train the lymphopoietic system, we employed an immunological triangle in which 3 animals are involved: a donor and a recipient of the same species, and an intermediate animal of a different species. The donor's tissue is used as an antigen to elicit anti-donor antibody in the intermediate species. The anti-donor antibody is then isolated and used as an antigen to elicit anti-antibody production in the eventual recipient. Such anti-antibody is intended to neutralize antibody produced by the recipient against the donor tissue.

To demonstrate this, we chose a simple type of homograft model based on blood transfusion in the dog. Since the canine type A erythrocyte elicits potent hemolysins in dogs of a type other than A, and since dogs do not possess naturally occurring anti-A isoantibodies[6] this system provided a readily available particulate antigen, easily assayed in vitro. We postulated that if the shape of the canine A red cell antigen determined the shape of the binding site of the canine anti-A antibody, it is probable that the same shape would prevail in anti-A produced in another species. If the anti-A binding site then became the antigenic determinant site and elicited anti-anti-A production in another individual, such anti-antibody A should bind any antibody having a binding site directed against the A red cell antigen.

This paper presents results achieved in application of the proposed immunological triangle to the training of the lymphopoietic system to produce anti-antibodies.

Materials and methods. Selection of animals. A dog whose blood exhibited strong hemolysis when tested against canine anti-A serum and strong agglutination against the indirect antiglobulin test was selected as the type A donor[6]. 5 dogs whose blood exhibited no hemolysis when tested against canine anti-A serum and were negative to the indirect antiglobulin tests, were considered non type A. 3 of them were used as recipients, and 2 as negative controls. 4 rabbits were used as intermediate animals for anti-A antibody production.

Antigens and antiserums. A 10% canine type A antigen suspension was used for stimulation of anti-A antibody formation in rabbits[7]. Rabbit anti-A serum was tested for hemolytic ability against canine type A cells. Rabbit anti-A antibodies were then isolated by adsorption to canine type A cells and then eluted from the cell surface by addition of 0.1 *M* sodium hydroxide[8]. The hemolytic ability was measured by substituting thus obtained rabbit anti-A antibodies for canine anti-A serum in the canine blood typing procedures[6]. Appropriate controls consisted of testing isolated rabbit anti-A antibodies against non-A type canine erythrocytes. Isolated rabbit anti-A antibodies were mixed with complete Freund's adjuvant and used for stimulation of anti-anti-A antibodies in the 3 non-A type dogs, to be recipients. The canine anti-anti-A serum was evaluated by determining its ability to inhibit hemolysis of type A erythrocytes by the rabbit anti-A serum and isolated rabbit anti-A antibody. To rule out non-specific inhibition, the control tubes prepared by mixing normal canine non type A serum with both rabbit anti-A serum and isolated rabbit anti-A antibodies, respectively, were titrated against type A erythrocyte suspension. The in vivo evaluation of this type of homograft model consisted of a 3 step procedure. Booster injections of isolated rabbit anti-A antibody were given to pre-treated recipient dogs 3 days before the first type A canine blood transfusion. Then transfusions of type A canine blood were given i.v. at 1 week intervals. Evaluation of the rejection reaction was performed by determining the ability of recipient dogs repeatedly transfused to hemolyze the donor type A blood. Two dogs other than blood A, not pre-treated for anti-

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anti-A production, served as negative controls. A massive transfusion, 200 ml whole blood, was attempted in 2 dogs: 1 pre-treated and 1 non-pretreated for anti-anti-A production.

Results. Hemolytic anti-A antibodies were produced in all 4 rabbits (1:32). After isolation, the pure anti-A antibody's hemolytic activity was 50% that of the rabbit anti-A serum (1:16). Canine anti-anti-A serum precipitated both rabbit anti-A serum and the isolated anti-A antibodies. All canine anti-anti-A serums precipitated with either rabbit anti-A serum or isolated anti-A antibodies produced no hemolysis when mixed with type A blood cell suspension. The hemolytic activity of rabbit anti-A serum or isolated anti-A antibodies was not impaired when normal canine serum was substituted for the canine anti-anti-A serum, indicating that the hemolytic activity was not impaired by normal serum components. After 12 transfusions, sera of 2 of 3 dogs pre-treated for anti-anti-A production lacked the ability to hemolyse type A erythrocytes, while sera of both the untreated dogs produced hemolysis. The dog not pre-treated for anti-anti-A production died of hemolytic reaction during the transfusion. The pre-treated dog survived the massive transfusion and exhibited no side effects, hemoglobinemia or hemoglobinuria. He is still alive and well.

Discussion. Anti-antibody was produced and its action demonstrated using the immunological triangle concept. The results achieved suggest that anti-anti-A antibody neutralizes anti-A antibody in vivo and prevents a demonstrable titer in hemolytic titration tests in vitro. The neutralization of anti-A antibody by anti-anti-A antibody has been shown to prevent the transfusion reaction in recipients pre-treated for anti-anti-A antibody production[9].

Zusammenfassung. Durch ein immunologisches Dreieck-System (Hund-Kaninchen-Hund) wurde ein Anti-Antikörper bei Hunden erzeugt und seine Wirkung in vitro und in vivo demonstriert. Die Anti-Antikörper-Methode bietet neue Möglichkeiten, dem Problem der Transplantat-Abstossung und der Therapie der Krankheiten immunologischen Ursprungs näherzukommen.

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Immunosuppressive and Specific Antimitotic Effects of Ovalicin

In a previous report[1] the immunosuppressive effect of ovalicin ($C_{16}H_{24}O_5$, isolated from cultures of *Pseudeurotium ovalis*[2]) in rodents was described: marked reduction of antibody production and of the number of antibody-forming cells in mice after stimulation with sheep erythrocytes and inhibition of the symptoms of experimental allergic encephalomyelitis in rats. Leukopenia was not observed in these experimental animals. It is the purpose of the present report to describe the effect of a single dose of ovalicin on primary and secondary hemagglutinin response in mice and on survival of skin grafts between mouse strains with identical H-2 locus. We have also investigated the effect of the compound on splenic weight in mice injected with red blood cells and/or with cells of L-1210 leukemia; furthermore, the frequency of mitoses after administration of ovalicin was evaluated in sections of spleen and jejunum of immunized and non-immunized animals.

Materials and methods. (Albino × DBA/2)F_1, BALB/c and DBA/2 male mice, 2–3 months old, were obtained from our own animal farm. 0.5 ml of a 10% suspension of washed sheep red blood cells (SRBC) in saline was administered i.v. for immunization. Hemagglutinin titres in sera were determined for each individual animal by serial 2-fold dilutions with Takatsy's microtechnic. L-1210 mouse leukemia cells were obtained from ascitic growth in (albino × DBA/2)F_1 mice; 10^6 cells were injected into the hind leg of experimental animals. Fitted 'pinch' grafts of skin from male DBA/2 donors were transplanted to male BALB/c recipients according to the method of Billingham and Medawar[3]. The survival time of the grafts was determined by daily inspection, the criterion being the endpoint of epithelial survival. For evaluation of the mitotic index in spleen and jejunum the organs were fixed in Bouin's fixative and stained with hematoxylin and eosin; the number of mitoses was then counted by a person who did not know from which animal the sections came. For each spleen, the mitoses in 150 fields, and for the jejunum mitoses/1000 epithelial cells in the crypts were counted (magnification × 1000). Ovalicin was administered as a suspension in 0.5% carboxymethylcellulose in saline either orally or i.p. The treatment schedules for each experiment are given with the results.

$$\text{S.D.} = \text{standard deviation} = s = \sqrt{\frac{\Sigma (x - x_i)^2}{n-1}}$$

Results. The primary hemagglutinin response in mice, as determined on day 7 or 14 after immunization with SRBC, was strongly inhibited by a single dose of ovalicin. Partial tolerance to the standard secondary challenge with SRBC was found when the primary response was inhibited with an oral dosage of 1200 mg/kg of ovalicin. When animals were treated with the drug on the day of the second challenge with SRBC only, there was but a slight inhibition of the response (Table I).

Table II shows the effect on skin graft survival in mice of a single dose of ovalicin, injected i.p. on different days

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