On the other hand, there was a statistically significant difference between the α coefficients ($P < 0.005$). The analysis permitted computation of the overall histometric variation of collagen fibres, the histometric variation within the groups and the histometric variation between the groups.

Discussion. A simplified semiquantitative histological method was used to study the relative frequency of collagen fibres in wound healing of alloxan diabetic rats.

Table IV. Test of linearity for the alloxan diabetic group

Source of variation	Sum of square	Degree of freedom	Mean square
Within groups	10,208.00	10	1,020.80
Regression	252,376.75	1	
about regression	5,798.25	3	1,932.75
Total	258,175.00	4	

$F_{(10;3)}$ observed $= 0.528 < F_{(10;3)}$ ($\alpha = 5\%$) $= 8.79$ (N.S.).

The values obtained for the rate of fibroplasia, have been proved to increase continuously in a linear fashion for both groups. On the other hand, the contents of total mature collagen fibres decreased throughout healing development in alloxan diabetic rats when compared to control animals. In the same way, the relative frequency of collagen fibres in an appropriate period of time seemed to be more reduced in diabetes than in control groups. On the other hand, the calculation for testing the difference between the regression coefficient showed that for each simple period the gross increase in collagen fibres was the same for both groups.

Résumé. La fréquence de la fibrillogenèse collagène dans le tissu de bourgeonnement augmente continuellement et sous une forme linéaire pour les groupes de contrôle et diabétique. D'autre part, le contenu de fibrilles de collagène dans une période de temps déterminée, semble plus réduite dans le groupe diabétique que dans le groupe de contrôle.

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Immunofluorescent Technique and Long Acting Thyroid Stimulator in Human Serum

Recently Blum et al.[1] have developed a rapid, highly sensitive assay for the detection of long acting thyroid stimulator (LATS), in human serum based on the immunofluorescent technique of Coons[2]. These authors have incubated guinea-pig thyroid sections first with human serum and then with fluorescein-labelled rabbit anti-human IgG γ-globulin. They have considered that a positive reaction, shown by a cytoplasmic fluorescence, was characteristic of the presence in the serum of an IgG γ-globulin with a particular affinity for the thyroid cell cytoplasm. They have established a correlation based on clinical and experimental grounds between a positive staining and the presence of LATS, detected by the McKenzie technique. They have differentiated this cytoplasmic reaction from the fluorescence due to the complement fixing antibody by the persistence of the staining after fixation of the tissues before assay, either in ethyl or methyl alcohol, fixation which destroys in human thyroids the complement fixing antigen[3].

The present study reports analyses performed on the sera of 22 thyrotoxic patients and 23 patients suffering from atrophic asymptomatic thyroiditis[4]. LATS was detected by the slightly modified McKenzie bioassay[5] and the complement fixing antibody by the indirect fluorescent Coons test[6]. The selected sera of thyrotoxic patients gave a bright cytoplasmic fluorescence with the Coons technique and displayed positive results in the LATS bioassay. The sera of the patients with atrophic thyroiditis were positive for the fluorescent detection of complement fixing antibody; in this latter group, no LATS assay was performed, but, in a previous work, we found only 4 positive reactions on 46 cases[7]. Thin sections (4–5 μ) of thyrotoxic human thyroid were fixed in methanol at 56 °C for 3 min, then incubated with the serum for 30 min, and afterwards for the same time with fluorescein labelled rabbit antihuman IgG γ-globulin[8]. Each incubation was followed by a washing with phosphate buffer at pH 7.2. The tissues were examined by UV-microscopy.

Neither in thyrotoxic nor in asymptomatic atrophic thyroiditis serum could any cytoplasmic fluorescence be detected.

A correlation has been established in thyrotoxic sera between the presence of LATS detected by bioassay and that of a positive cytoplasmic fluorescent reaction with unfixed human thyroid sections. However, a lot of sera showed diverging results when tested by both methods[9]. These data suggest that these 2 techniques do not detect the same antibody in the serum.

The disappearance of any cytoplasmic fluorescence after methanol fixation of the tissues agrees with the data

[1] A. S. Blum, F. S. Greenspan, J. H. Hargadine and J. M. Lowenstein, Metabolism *16*, 960 (1967).
[2] A. H. Coons and M. H. Kaplan, J. exp. Med. *91*, 1 (1950).
[3] I. M. Roitt, N. R. Ling, D. Doniach and K. G. Couchman, Immunology *7*, 375 (1964).
[4] P. A. Bastenie, P. Neve, M. Bonnyns, L. Vanhaelst and P. Chailly, Lancet *1*, 915 (1967).
[5] M. Bonnyns, Revue fr. Étud. clin. biol., in press (1968).
[6] E. J. Holborow, P. C. Brown, I. M. Roitt and D. Doniach, Br. J. exp. Path. *40*, 583 (1959).
[7] P. A. Bastenie, M. Bonnyns and L. Vanhaelst, in *Proceedings of Thyrotoxicosis Symposium* (Ed. W. J. Irvine; Livingstone, London 1967), p. 40.
[8] B. M. Balfour, D. Doniach, I. M. Roitt and K. G. Couchman, Br. J. exp. Path. *42*, 307 (1961).
[9] M. Bonnyns and L. Vanhaelst, J. Endocr., in press (1968).

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-

1 F. MILGROM, S. DUBISKI and G. WOZNICZKO, Vox Sang. *1*, 172 (1956).
2 F. MILGROM and S. DUBISKI, Nature *179*, 1351 (1957).
3 H. G. KUNKEL, M. MANNIK and R. C. WILLIAMS, Science *5*, 140 (1963).
4 J. B. NATRIG, Acta. path. microbiol. scand. *65*, 559 (1965).
5 A. S. KELLUS and G. H. GELL, J. exp. Med. *30*, 1 (1968).
6 S. N. SWISHER and L. E. YOUNG, Phys. Rev. *41*, 3 (1961).
7 D. H. CAMPBELL, J. H. GERVEY, N. E. CREMER and D. H. SUSSDORF, in *Methods in Immunology* (W. A. Benjamine, New York 1964), p. 71, 101.
8 J. B. KWAPINSKI, in *Methods of Serological Research* (Wiley and Sons, New York 1965), p. 118.