of a surface receptor on the HRC. For this purpose, several blood samples have been selected according to their behaviour in the IAH (strong, weak, negative), and attempts have been made: a) to adsorb the IAH components (antigen-antibody complex and complement) comparatively on strong, weak and negative HRC; b) to remove the presumable receptor from the positive HRC and to use it as an inhibitor of the IAH; c) to immunize rabbits with HRC of different reactivity and to prepare specific antibodies to the presumable receptor.

a) In the first group of experiments, blood samples with different reactivity were incubated with the antigen-antibody complex (chick fibroblast extract-rabbit anti-chick fibroblast serum) in presence of complement as for a regular IAH test. After 30 min at room temperature, supernatants were removed by centrifugation and were used to perform again an IAH reaction by adding them to fresh untreated HRC, known to be strong positive in IAH. When added to the fresh strong positive HRC, supernatant removed from strong positive HRC generally gave negative results or very low titers; supernatants removed from weak HRC gave titers lower than before incubation; meanwhile snpernatant removed from negative HRC allowed high titers of IAH. Similar results were obtained when ghosts prepared from blood samples with different reactivity (strong, weak, negative) were incubated with the antigen-antibody complex in presence of complement, and supernatants, removed for subsequent IAH with fresh untreated HRC known to be positive in IAH.

b) In order to remove the presumable receptor, trypsinization of different HRC was performed, and the HRC used further in IAH reactions. Strong positive HRC became negative for IAH, after trypsinization, meanwhile negative ones were not influenced by this treatment. Supernatants obtained by trypsinization of positive HRC, and added to an antigen-antibody complex in presence of complement, were able to inhibit the IAH with known positive HRC. Supernatants removed from trypsinized negative HRC did not inhibit the IAH.

c) Rabbits have been immunized, respectively, by a single inoculation with strong positive and negative HRC from different donors and with ghosts prepared from these HRC. Sera were withdrawn every day between 5–9 days after inoculation, so as to avoid formation of a large amount of anti species antibodies and to obtain, as far as possible, antibodies directed chiefly against the surface receptor. These sera were inactivated by heating at 56 °C for 30 min; samples from every sera were adsorbed on HRC known as negative in the IAH, so as to remove the anti HRC antibodies. Haemagglutinating properties of nonadsorbed and adsorbed sera were checked comparatively with positive and negative HRC from different donors. Sera from rabbits immunized with positive HRC showed relatively high titers of haemagglutination with positive HRC and low titers with negative HRC; after adsorption on negative HRC, these sera lost their ability to agglutinate negative HRC, but kept their agglutinins for positive ones. Sera from rabbits immunized with negative HRC had low titers of haemagglutination equal for both positive and negative HRC; after adsorption on negative HRC, their agglutinins decreased equally for positive and negative HRC.

Sera from rabbits immunized with ghosts prepared from positive and negative HRC gave results similar to those obtained with sera from rabbits immunized with fresh HRC. It is worth noting also that sera from rabbits immunized with positive HRC and adsorbed on negative HRC were able to agglutinate microscopical fragments obtained by supersonic vibration treatment of stroma prepared from positive HRC.

Our results lead to the conclusion that a surface receptor characterizes the HRC which are suitable for the IAH. This receptor, which can be removed by trypsine, seems to be abundant on strong positive HRC, while only small amounts are present on the weak HRC.

We do not know as yet whether this receptor has a biological role or whether it is only a genetic marker of the HRC[10].

Résumé. Trois types d'hématies humaines sont décrites (fortes, faibles, négatives) selon leur réactivité dans l'adhérence sérologique (agglutination d'hématies humaines, exposées à un couple antigène-anticorps, en présence du complément). Ces différences individuelles sont dues à un récepteur dont la présence sur les hématies positives, est demontrée directement (adsorbtion des réactifs sur les hématies positives) et indirectement (perte de la réactivité par trypsinisation, inhibition de la réaction et préparation de sérums spécifiques anti-récepteur).

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Laser-Induced Excitation of Fluorescein Isothiocyanate in the Immunofluorescence

In recent years immunofluorescent procedures have been increasingly used in basic as well as in applied immunology. This was to be expected, since the immunofluorescence method is extremly sensitive. BARTELS[1] reported that quantities as small as 10^{-18} g of fluorescein isothiocyanate (FITC), roughly corresponding to 1000 molecules, could be detected by this method. So far only radioisotope tracers could be detected in such small concentrations.

At present it is not feasible to take full advantage of this method. There are still many facets which have not been solved. Let us mention only two of them: the effi-

cient excitation of FITC involving the light source and the construction and matching of the excitation and barrier filters, and the system antigen-antibody-conjugate.

In the present communication, recent developments in the excitation of FITC in immunofluorescence are touched upon and experiments with laser excitation of FITC in immunofluorescence are reported.

Recently descriptions have been published of interference filters with high transmittance in the region of maximum FITC absorption and with a particularly steep slope toward 500 nm, the transmittance at 525 nm being 0.1–0.001% (KRAFT[2], RYGAARD and OLSEN[3]).

They permit an efficient excitation of FITC with a minimum of unwanted fluorescence by means of a halogen or a tungsten lamp, a fact greatly facillitating the routine work in immunofluorescence.

If visible light is used for FITC excitation, a barrier filter should be used to exclude the strong excitation light and to make fluorescence observable. Usually this is a coloured glass filter or dielectric multilayer filter which opens steep to the emission of FITC. Since the absorption peak at 490 nm and the emission peak of FITC at 520 nm are only 30 nm apart, it is difficult to match the excitation and the barrier filter. Ideal conditions would be obtained if we had an excitation filter whose transmittance fell vertically at 500 nm and a barrier filter which opened vertically at the same wave-length. No such filter system has been designed, but very good results have been obtained with the interference filter combined with Zeiss 50 barrier filter (RYGAARD and OLSEN[3]) and also an interference filter KP 490 combined with a K 510 filter (KRAFT[2]).

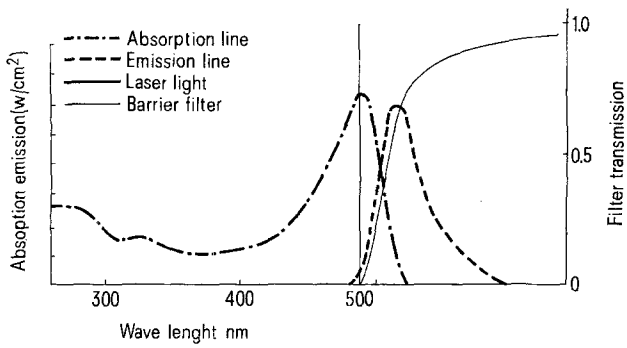

Fig. 1. Spectral distribution of absorption and fluorescence of FITC, laser light and filter transmittance.

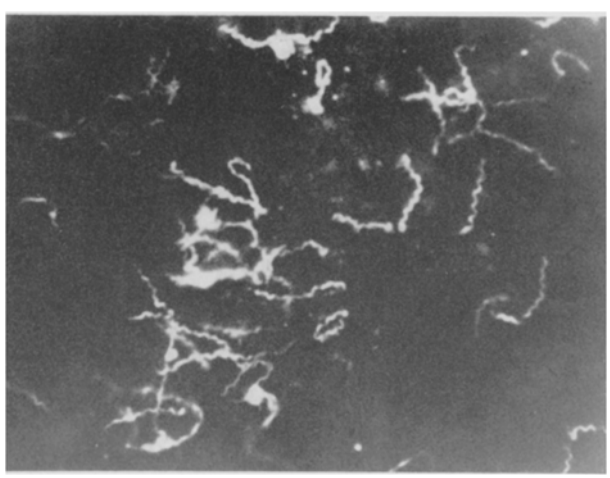

Fig. 2. Fluorescent treponemes in a positive FTA-ABS test.

In our experiments, a monochromatic light beam at 488 nm from an argon laser was used instead of conventional light. The advantages of the system with laser light source are obvious. The wave-length almost coincides with the FITC absorption peak. Since the laser light is monochromatic, there is no need for an excitation filter and the appropriate barrier filter can easily be selected. In our hands the Zeiss 50 barrier filter gave good results.

The argon laser we worked with was made by E. VRENKO and I. RAJVER of the Department of Technical Optics, Iskra, Ljubljana. The energy of 100 mW was sufficient to excite bright fluorescene. We used the standard Reichert Zetopan microscope with a dark-field condenser from which the lamp, lenses and filters were removed. Since the laser beam was too narrow, an objective of 40× magnification was inserted between the laser and the condenser to enlarge the beam. In this way enough light reached the object through the annular construction of the dark-field condenser. The microscope image obtained in this way was clear and sharp.

We employed this system in the FTA-ABS test for syphilis. In negative specimens neither treponemes nor any other tissue or material were detectable. In positive sera, the titers were always much higher than those obtained by standard method. Very little autofluorescence was observed. The specificity and sensitivity of the laser induced FTA-ABS test for syphilis is now being investigated.

Zusammenfassung. Methode für die Anregung des FITC in der Immunofluoreszenz mit Argon-Laser-Licht, die eine sehr starke Fluoreszenz ermöglicht. Diese Methode wird in dem FTA-ABS-Test für Syphilis benutzt.

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The Induction of Ovarian Dysfunctions in *Thermobia domestica* by the *Cecropia* Juvenile Hormones

The corpora allata, source of the juvenile hormone, have long been known to stimulate insect reproduction by supporting the yolk deposition into the growing oocytes[1]. The juvenile hormones isolated from *Hyalophora cecropia* were also shown to possess this stimulatory activity[2,3]. We have found, however, that administration of 1–100 µg of either of these 2 hormones[4] into an adult female of the firebrat *Thermobia domestica* Packard, may, in a long run,