# STUDY OF THE ORGAN-SPECIFICITY OF HUMAN THYROID TUMORS

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Since the first examples of "antigenic simplification" of tumors were described some 10 years ago, several reports have been published confirming this observation [2, 4, 11, 12]. The essence of the phenomenon is that certain antigens found in the tissue from which a tumor is derived are not present in the tumor itself. When investigators have tried to determine the nature of the lost antigens, with rare exceptions they have found that they are organ-specific [1, 2, 12].

The object of this investigation was to study the "antigenic simplification" of tumor arising from tissues with marked organ-specificity. Tumors of the thyroid gland were chosen as test objects for the additional reason that very little work has been done on the study of human tumors in this respect.

## EXPERIMENTAL METHOD

The tumors were obtained from the operating rooms of our institute and normal tissues in the mortuary from the cadavers of persons dying from trauma. The material was kept at -15°. As antigens for immunization and for the reactions saline extracts (1 part of tissue to 3-4 parts of physiological saline) of the tumor and normal tissues were used.

Different schemes of immunization were carried out. Usually the first cycle of immunization consisted of 6 injections of increasing doses of antigen at 3-day intervals. Initially a dose of 1.0-1.5 ml was injected intravenously, and this was followed by 2-3 intraperitoneal injections (1.5-2.5 ml); finally, 2 intramuscular injections were given, each in a dose of 3.0-3.5 ml. Blood was taken 7-8 days after the completion of the cycle. One month later reimmunization was carried out by means of 2 intramuscular injections of 3 ml of antigen at an interval of 1 week. Several reimmunizations were given: the most active sera were usually obtained after 2-3 cycles of immunization. The titer of the sera was often increased by concentrating them with ammonium sulfate [4].

Almost invariably, exhausted sera were used, for which purpose different exhausting antigens were added to the serum under the control of Ouchterlony's reaction. Very often Bjerklund's method also was used [6], in which the exhausting antigen was added to the agar before the reaction. Ouchterlony's reaction was carried out in the modification usually practiced in L. A. Zil'ber's laboratory [4]. The antigens used for the reactions were usually balanced in respect of their protein content or the difference in the protein content was taken into account, its level being determined either by the micromodification of Kjeldahl's method or by Kingsley's method (with biuret).

The simplest form of fractionation of the saline extracts from the thyroid was performed with ammonium sulfate. A saturated solution of ammonium sulfate was added to an equal volume of extract of one of the glands (1 part of tissue to 4 parts of physiological saline). In the solution thus formed, with 50% saturation, the thyroid globulins were precipitated and the albumins remained in solution. After separation of the fractions by centrifugation, they were dialyzed against veronal-medinalbuffer and tested in the immunoelectrophoresis reaction, using the modification of Abelev and Tsvetkov [4]. Electrophoresis was carried out at a potential gradient of 10 V/cm for 1 h. The reaction was developed by addition of either untreated or exhausted anti-thyroid serum.

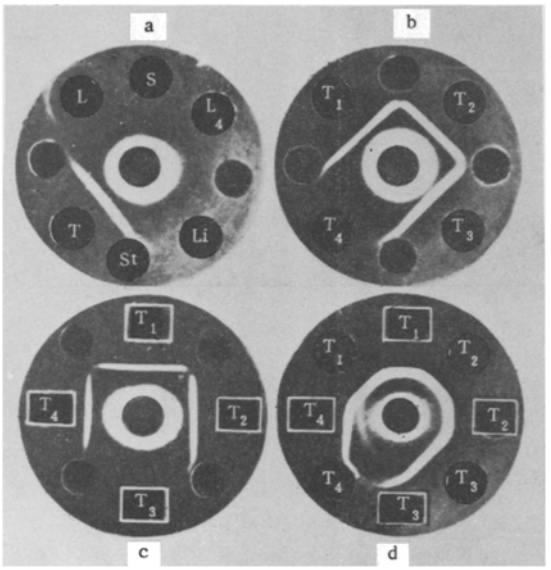


Fig. 1. Reaction of serum against thyroid tissue, neutralized with human lung antigens, and with antigens from normal organs and thyroid tumors. a, b, c, d) Variants of the reaction. Serum—in central well; antigens—in peripheral wells. Identity of antigens: L) lung; S) spleen; K) kidney; Li) liver; St) stomach; T) thyroid; $T_1$, $T_2$, $T_3$, etc.) antigens from tissue of thyroid tumors.

## EXPERIMENTAL RESULTS

In most cases the sera obtained as a result of immunization or rabbits with thyroid extract possessed fairly high organ-specificity. Some of the untreated sera obtained after the first cycle of immunization gave a clear reaction only with antigens of the thyroid, and gave either no reaction whatever or hardly visible lines of precipitation with other organs. However, most of the sera gave reactions with antigens of other organs and with serum proteins. Particularly strong nonspecific reactions were given by sera obtained after late cycles of immunization, and also by concentrated sera. After addition of the corresponding antigens, the nonspecific antibodies could be neutralized fairly easily and a monospecific serum against thyroid tissue could be obtained.

The reaction between one such serum, neutralized with human lung antigens, is shown in Fig. 1, a. The serum gave a clear line with thyroid antigen but did not react with antigens from the spleen, kidney, liver, gastric mucous membrane, and lung of the same cadaver from which the thyroid was taken.

Organ-specific sera against the thyroid reacted very well with antigens from other thyroids (Fig. 1, b). Of the 22 glands studied, only 2 did not react with the organ-specific sera.

The results of the immunoelectrophoresis reaction carried out with thyroid extract and its fractions may be seen in Fig. 2. The albumin fractions gave several very weak lines in different zones. Lines in different zones were also given by untreated thyroid extract with untreated anti-thyroid serum, the reaction being most marked in the zone of the $\alpha$-globulins. The same extract with an exhausted organ–specific serum gave one clear line in the same zone. The globulin fraction of the thyroid likewise gave a single line in the zone of the serum $\alpha$-globulins.

Consequently, specific thyroid antigen is precipitated by ammonium sulfate in 50% saturation and is detected by the immunoelectrophoresis reaction in the zone of the serum $\alpha$-globulins. These findings show that this particular organ-specific antigen is thyroglobulin [10]. Thyroglobulin gave a much weaker reaction than the untreated antigen. The salting-out procedure must have brought about some degree of denaturation of this substance.

In the immunoelectrophoresis reaction (Fig. 2) thyroid extract heated for 30 min at 100° was tested. The extract had completely lost its power of reacting with anti-thyroid serum. In face of the discovery, made by Milgrom and co-workers [6], of the high thermostability of thyroglobulin, we carried out a few experiments with other thyroid antigens. These showed that all the tested thyroid extracts completely lost their ability to react with anti-thyroid serum after heating in this way.

On the assumption that this was the result, not of destruction of the antigen, but of its conversion into an insoluble form we carried out experiments to test the specific absorption of anti-thyroid sera with antigens heated to 100°. These showed that antigens heated for 20 or 30 min lose their ability to absorb antibodies, whereas extracts heated for 10 min, and more especially for 5 min, retain this ability to a considerable degree. Hence, these results differed from those obtained by the authors cited above [6], in whose words boiling the antigens for 30 min "had absolutely no effect on them." The difference in the results can evidently be attributed to the fact that we tested extracts, while Milgrom and co-workers tested a suspension of thyroid gland tissues, in which the surrounding structures in the particles of the gland probably prevented destruction of the thyroglobulin. Furthermore, the possibility of the existence of an insoluble, thermostable form of thyroglobulin cannot be ruled out.

In Ouchterlony's reaction we noted that in some cases the organ-specificity of the thyroid was marked by more

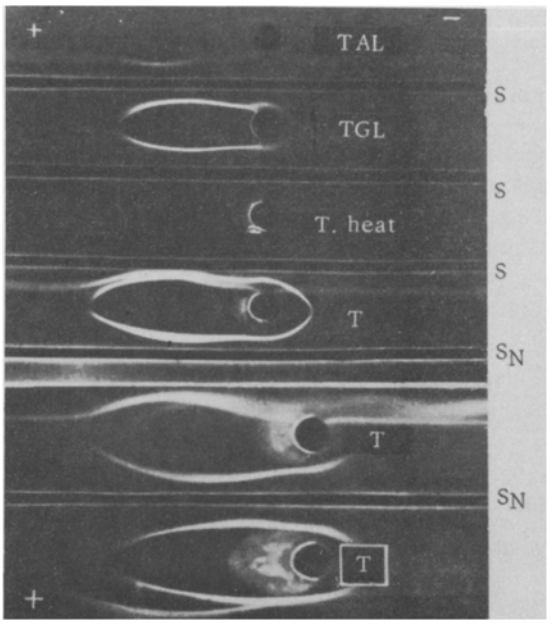


Fig. 2. Reaction of immunoelectrophoresis with thyroid extract and its fractions. Legend: TAL) albumin fraction of thyroid; TGL) globulin fraction of thyroid; T. heat) thyroid extract heated for 30 min to 100°; S) untreated anti-thyroid serum; $S_N$) organ-specific anti-thyroid serum. Remainder of legend as in Fig. 1.

than one line. When the reaction was performed with serial dilutions of antigens, it was found that the organ-specific thyroid antigens most frequently gave several lines of precipitation (Fig. 3). We attempted to separate the antigens in the immunoelectrophoresis reaction, but this proved impossible for these antigens are distinguished by their very slow movement in an electrical field. Hence, our results confirm those obtained by other authors [8, 9] in respect to the complex nature of thyroglobulin. As Ouchterlony's reaction showed, this substance consists of at least three antigenic components.

We also tested antigens from various malignant and benign thyroid tumors in reactions with organ-specific anti-thyroid sera. As a rule these also gave positive reactions. Of the 4 tumor antigens, 3 gave equally clear precipitation lines with anti-thyroid serum as the antigens of the normal glands (Fig. 1, c). Of 26 thyroid tumors tested, only 2 failed to show the presence of organ-specific antigens. It might be supposed that the presence of organ-specificity in the tumors was attributable to contamination of the test samples with normal gland cells. However, organ-specific antigen was found, not only in the primary tumors, but also in the metastases. Of the 7 metastases investigated, organ-specific antigen was found in 6.

Organ-specific thyroid antigen was also demonstrated by antiserum against thyroid carcinoma. This anti-carcinoma serum, when exhausted by addition of human serum, gave a clear reaction only with thyroid antigen; no reaction or a hardly visible reaction was given with antigens from other organs.

Evidently the organ-specific antigens of the thyroid gland and its tumors were the same or very similar substances. This could be concluded from the fact that a common line of precipitation—a line of identity—was formed in Ouchterlony's reaction with alternate arrangement of the tumor and normal antigens (Fig. 1, d). Extract of thyroid carcinoma gave a line with the organ-specific serum in the immunoelectrophoresis reaction (Fig. 2) which took the form of a mirror-image of the line formed in the same reaction by extract of the normal thyroid gland. Further evidence in support of this conclusion was also given by the fact that organ-specific antithyroid sera were completely exhausted by addition of antigens from metastases of thyroid carcinoma.

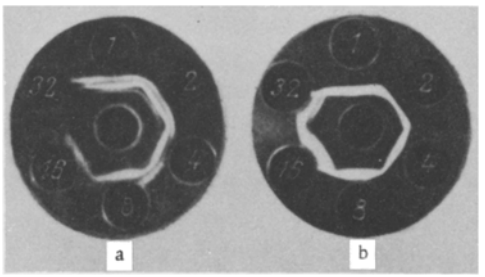


Fig. 3. Reaction of organ-specific anti-thyroid serum. a) With serial dilutions of tissue antigens of thyroid carcinoma; b) of normal thyroid gland. Numbers denote dilutions of antigens.

Hence, in a wide variety of benign and malignant thyroid tumors, an organ-specific antigen soluble in physiological saline was readily demonstrated by the precipitation reaction in agar. These results are in sharp contrast to the previous findings with carcinoma of the stomach [3]; in that case in the same experimental conditions no organ-specific antigens could be found in the tumors. "Antigenic simplification" of tumors is a process which varies in nature in different tumors, affecting different antigens in different cases, as has been shown so clearly in the mouse hepatomas [1].

Nevertheless, it cannot be concluded from these results that "antigenic simplification" does not in general take place in thyroid tumors. By the methods used, only the soluble antigens could be studied. Beutner's findings show that "antigenic simplification" of the thyroid may be demonstrated by the method of labeled antibodies [5]; similar conclusions may be drawn from the findings of Witebsky and co-workers [13], using the complement fixation reaction.

Hence, we did not find complete loss of soluble organ-specific antigens (thyroglobulin) by thyroid tumors. The problem of possible quantitative changes in their content requires further investigation.

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**All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.**

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