Med. Microbiol. Immunol. 161, 133–144 (1975)

Isolation and Some Serological and Epidemiological Data on the Viruses Recovered from Patients with Subacute Thyroiditis de Quervain

D. Stanček[1], M. Stančeková-Gressnerová[2], M. Janotka[3], P. Hnilica[4], and D. Oravec[5]

[1]Regional Hygiene Station, Regional Institute of Health,
[2]Chair of General and Applied Virology, Faculty of Natural Sciences, Commenius University,
[3]Divisions of Radioisotopes, Department of Medicine, Medical Faculty Hospital,
[4]Postgraduate Medical Institute, Department of Medicine,
[5]Endocrinological Station, Regional Institute of Health, Bratislava

Received January 9, 1975

Abstract. Virological and serological methods were used in examination of 28 patients suffering from subacute thyroiditis de Quervain. Attempts to isolate a presumed viral agent from 8 patients were performed by inoculation of serum, urine, and aspiration biopsies of thyroid glands taken at different stages of the illness, into tissue cultures of different types of human and animal cells. Recovery of a cytopathic viral agent on cells of a rabbit lung continuous line was successful in 5 cases. Serological cross reactions exist between the isolated viruses and patient serum but not with serum of healthy people. Cases with the acquired illness and positive antibodies against the isolated viruses who had been in close and prolonged contact with patients suffering from subacute thyroiditis de Quervain were also investigated.

Introduction

Inflammatory infections of thyroid gland are not a rare form of pathological disturbance in this organ. They have often occurred in limited epidemics (Green, 1971; Janotka *et al.*, 1974) or following acute inflammatory infections of the upper respiratory tract such as mumps, measles, infectious mononucleosis, influenza, and others (Werner and Ingbar, 1971). It is presumed that their incidence has shown a tendency to increase in the last years (Woolner *et al.*, 1962). The etiology of these inflammations, however, remains an unresolved question. Among infectious agents, viral origin of the thyroid gland inflammations has only been studied rarely, though viruses are often suspected to be responsible for the acute or more or less prolonged inflammatory affections of this organ. Paramyxoviruses, such as the mumps virus, have often been considered to have a causal relationship with thyroiditis de Quervain (Eylan *et al.*, 1957). Other reports pointing to an etiological relationship between myxo- or paramyxoviruses and subacute thyroiditis are based on indirect, mostly serological or epidemiological, data (Volpé *et al.*, 1967; Felix-Davis, 1958). In one case of chronic thyroiditis and thyroid carcinoma, herpes-like particles and particles resembling oncogenic-type C viruses were seen by electronmicroscopy in a buffy-coat cell line derived from a patient (Maruyma *et al.*, 1968).

By inoculation of rabbit lung cells with materials from a patient suffering from subacute thyroiditis de Quervain we succeeded at the end of 1972 in isolating a so far unclassified virus (Stanček and Gressnerová, 1974). In the present report we describe further results of our virological, serological, and in part epidemiological studies concerning the etiology of subacute thyroiditis de Quervain.

Materials and Methods

Tissue Cultures. Rabbit lung cell cultures (RLC) (Szanto, 1960) were supplied by the tissue culture laboratory of the Institute of Virology, Slovac Academy of Sciences. The cells were cultivated in Earle's medium enriched with glucose, yeastolate, 5 to 10% heat-inactivated calf serum (SEVAC, Prague), and antibiotics. The usual concentration of cells grown in Roux bottles or glass tubes was about 2×10^5 cells per 1 ml of media. A permanent line of hamster kidney cells (BHK), also supplied by the tissue culture laboratory of the Institute of Virology, was cultivated in Eagle's Basal Medium, supplemented with 10% of heat-inactivated calf serum and antibiotics. The cell concentration was the same as that with RLC. Cultures of human diploid cells obtained regularly from the Institute of Sera and Vaccines in Prague were kept in EPL medium (SEVAC, Prague) with 10% calf serum and antibiotics.

Titrations of the Isolated Viruses. The viral isolates adapted to RLC cultures were titrated either by inoculation of newly transferred cultures of RLC or by inoculation of the infectious materials on 2 to 3 day-old monolayers of RLC; 3 to 4 glass tube cultures were always inoculated with one dilution of virus. The infected cultures were incubated at 35°C or 37°C for 3 to 5 days. Results were read according to the cytopathic effect (CPE) of the virus consisting of syncytia formation which, at a sufficient multiplicity of infection, were large and well separated, resembling plaques. The reciprocal of highest dilution of the inocula giving rise at least to 2 to 3 syncytia in each of the parallel cultures was considered to be the titer.

Virus Neutralization Tests. 0.4 ml of patient serum or control serum diluted 1:2 or 1:10 were mixed with 0.4 ml of one of the isolated viruses designated as the "MGI strain". The titer of the virus was about 400 CPD_{50} per 0.4 ml. The mixture was incubated at 35°C for 90 min. After incubation 0.2 ml of the mixture was transferred into tubes with $8 \times 10^4 - 1.2 \times 10^5$ RLC. The treated cultures were then incubated at 37°C or 35°C. At the same time uninfected control cultures and cultures infected with the same dose of the virus used in neutralization tests were prepared. Results were usually read between the 4th and 8th days of incubation. Calculations were done by comparing the number of "plaques" formed by syncytia in the infected control cultures and the cultures treated with mixtures of serum and virus. Only those sera were considered positive which in a given dilution neutralized at least 50% of the control "plaques". We counted average amounts of plaques in 5 to 10 microscopical fields of each of the 2 to 4 parallel cultures.

Indirect Immunofluorescence. 1 to 5-day-old RLC cultures infected with MGI strain or uninfected control cultures grown on cover slips (8×20 mm) placed in glass tubes were rinsed with buffered saline solution, pH 7.2 (BSS), dried and fixed in distilled acetone for 10 min at room temperature. The fixed cover slips were used either immediately or stored at −20°C for several weeks. Fixed cells were rinsed before use with BSS and 0.1 to 0.15 ml of serum diluted 1:2 or 1:10 in BSS was added. The cultures were then incubated for 45 min at 35°C in a moist chamber. After thorough washing in BSS, cultures were treated with 0.1 ml of fluorescein-labeled swine globulin against human globulin (SwAHu/FITC, SEVAC, Prague) and kept for 45 min at 35°C in a moist chamber. After staining, the cultures were washed 3 times for 20 min in BSS and once in redistilled water. Finally, cover slips were mounted in glycerol-Tris buffer solution pH 8.0. Fluorescent microscope Fluoval, Carl Zeiss, Jena, was used for observation of results.

An indirect immunofluorescent test with sera to test for the presence of antibodies against Epstein-Barr virus capsid antigen (EB-VCA) was carried out according to the method described by Henle *et al.* Lymphoblastoid cell lines derived from patients with Burkitt's lymphoma were kindly supplied by Dr. J. Werner from the Robert Koch Institute in West Berlin.

Results

Isolation of Viruses from Patients with Inflammatory Affections of the Thyroid Gland

Samples of blood, urine, and, when possible, also aspiration biopsies of the glands were taken from patients with subacute thyroiditis de Quervain during the first days of hospitalization (5—30 days of the illness) or later as needed. Samples were prepared with antibiotics. After low-speed centrifugation the samples were added to suspensions or 1 to 2-day-old monolayer cultures of RLC cultivated in Roux bottles or glass tubes. The cultures were checked thereafter microscopically every day for the appearance of cytopathic changes. After 2 to 5 days of incubation the culture media were replaced. Passages were always carried out regardless of the presence or absence of CPE after 6 to 8 days of incubation. When more intensive CPE appeared the infection of the cultures between two passages was maintained by addition of fresh non-infected RLC. In some cases "blind passages" of virus were carried out by trypsinization and seeding of the infected cells into new bottles or glass tubes with an admixed portion of fresh non-infected RLC.

Among tested materials taken from 8 patients admitted to local hospitals, viral agents which were cytopathic after adaption for RLC or other animal or human cells were recovered in 5 cases. The agent could be recovered from serum, urine, and aspiration biopsies of the thyroid gland (Table 1). Reisolation attempts carried out with patients (M.G., M.L.) during relapses of thyroiditis which occurred several months after the first attacks were successful and yielded the same viral agent. In the case of patient M.G., repeated health difficulties were observed after the first attack of thyroiditis de Quervain. The patient showed

Table 1. Positive isolations of viruses from patients with subacute thyroiditis de Quervain

Patient, age, sex	Diagnosis	Samples taken (data)	Virus isolated from	Notes
M.G., 34 years, female	Thyroditis subacuta de Quervain	11. Dec. 1972 14. Dec. 1972	serum, urine thyroid gland, aspiration biopsy	Reisolation of the viral agent from original materials possible
Same as above	Lymphadenitis cervicalis lat. sin. Relapse with the same etiology?	3. Aug. 1973	serum	Reisolation of the same virus 7 months after the 1st attack
M.L., 26 years, female	The 2nd attack of thyroiditis subacuta de Quervain	17. Oct. 1973	serum	Relapse 10 months after the 1st attack
M.H., 35 years, female	Thyroiditis subacuta de Quervain	16. Jan. 1974	thyroid gland, aspiration biopsy	
H.M., 47 years, female	Thyroiditis subacuta de Quervain	18. Jan. 1974 13. Feb. 1974	serum, urine serum, urine, thyroid gland biopsy	
S.N., 35 years, male	Thyroiditis subcuta de Quervain	13. March 1974	serum, urine	

lymphadenopathy of the cervical and inguinal lymph nodes, subfebrile temperatures, and a weakness lasting several weeks. 7 months after recovery from the initial attack, during the period of the above-mentioned difficulties, we succeeded in reisolation of a virus serologically and cytopathogenically similar to the virus isolated from the first inflammation of thyroid gland. In the case of patient M.L. there was no opportunity for virus isolation during the first attack of clinically confirmed thyroiditis de Quervain. 10 months later, however, the patient had the second typical attack of subacute thyroiditis during which the virus, serologically and cytologically similar to the other described viral isolates, could be recovered. All 6 isolates induced rather similar cytopathological features and showed serological cross reaction.

The first appearance of CPE of the isolated viruses was identical in all cases: decreased metabolism of the infected cells, monolayers with spots of rounded cells, and formation of multinuclear giant syncytial cells later in the process of adaption of the virus to RLC cultures. Large syncytia could be found, however, only 2 to 7 weeks after the original inoculation of cultures with patient materials. In some patients (M.G., M.H., S.N.), viral strains cytopathic for RLC, BHK, human diploid cells, and other cell cultures could be isolated by repeated passage. The titers of the adapted viruses were usually 10^3 to 10^5 CPD_{50} per ml. Titers of the viruses grown on BHK or human diploid cells were regularly somewhat higher. On human diploid cells, however, the infectious virus could be reproduced only up to 10 to 15 passages after which it disappeared from cultures. The reason for this remains obscure.

Time Course of Neutralizing Antibody Production in the Patient

Neutralization tests were performed using the virus isolates and consecutive serum samples obtained from one patient (M.G.). The same samples had been used for indirect immunofluorescent tests. For both tests the serum taken at the beginning of the illness was negative (Table 2). In the second sample, taken 2 days later, virus neutralization activity as well as immunofluorescent staining remained absent. The serum taken 3 weeks after the beginning of the illness was slightly positive after examination using immunofluorescence. Virus-neutralizing antibodies were first observed in the serum sample received $2^1/_2$ months after the onset of thyroiditis. These antibodies were present in the patients' serum for at least 1 year. Table 2 presents a short account of the course of the illness, therapy used, and isolations of viruses.

Virus Neutralization Tests with the MGI Strain of the Isolated Virus Using Patients' Serum and the Serum of Healthy Individuals

Some results of serological tests with various sera are presented in Table 3 and Table 4. Serum was obtained from 28 patients hospitalized with a diagnosis of subacute thyroiditis de Quervain, one patient with chronic thyroid gland disorders, and 15 healthy donors. The results show that in the group of patients with thyroiditis de Quervain, 15 sera (53.5%) were highly positive, 5 sera (18%) were slightly positive, and 8 sera (28.5%) were negative. On the other hand, none of the sera taken from 15 healthy people was highly positive, although one serum was slightly positive and one moderately positive. 13 sera (86%) were negative. The serum from the patient with chronic thyroid disorders was moderately positive.

Table 2. Clinical course of the illness and production of MGI strain-neutralizing and immunofluorescent antibodies in sera of a patient with thyroiditis subacuta de Quervain (M. G., 34 years)

Date	Clinical course of the disease	Results of NT with MGI strain and patient's serum	Immunofluorescent test with MGI strain-infected RLC and patient's serum	Notes
1. Dec. 1972	The beginning of the illness, headache, T 37.5°—40.2°C	n.d.	n.d.	Treatment at home with antipyretics, vitamins
11. Dec. 1972	Intensive pain and local skin inflammation over thyroid region, T 38.5°—40.0°C	negative	n.d.	Hospitalization with dg.: Infectious mononucleosis. Antipyretics, Tetracyclin
13. Dec. 1972	After Prednison therapy T back to normal	negative	negative	Final dg.: Thyroiditis subacuta de Quervain. Prednison
14. Dec. 1972	Unchanged	n.d.	n.d.	Positive isolation of the virus
23. Dec. 1972	Further improvement	n.d.	slightly positive	Released from hospital. Prednison therapy continues
16. Jan. 1973	Without difficulties	slightly positive	positive	Prednison therapy continues
31. Jan. 1973	Without difficulties	n.d.	n.d.	Prednison therapy stopped
February-March 1973	Regular headaches and subfebrile temperature	highly positive	positive	Antipyretics
7. June 1973	Without difficulties	highly positive	n.d.	
3. Aug. 1973	Lymphadenitis cervicalis lat. sin., T 37.0°—37.6°C. Headaches	highly positive	positive	Checking in hospital. Antipyretics. After 2 weeks back to normal. Virus isolated
15. Nov. 1973 up to now	Without difficulties	moderately positive	n.d.	

n.d. not done.

Table 3. Neutralization effect of some of patient sera on the isolated virus

Patient serum[b]	Number of "plaques" per 1 ml inoculum[a]	Size of "plaques"
Negative control	1.58×10^2	large
T.K.	1.4×10^1	small
M.T.	2.7×10^1	small-medium
J.B.	8.4×10^1	large
J.J.	2.3×10^1	small-medium
M.G.	1.2×10^1	small
H.V.	0.8×10^1	small
J.M.	1.4×10^1	small-medium
L.S.	8.2×10^1	large
J.J.	3.1×10^1	medium
I.B.	6.4×10^1	large
I.H.	5.4×10^1	medium
A.M.	2.9×10^1	medium
M.B.	8.6×10^1	medium
Z.H.	6.9×10^1	medium-large
Z.M.	1.08×10^2	large

[a] Greater than 50% neutralization of virus by patient sera was considered "positive".
[b] Dilution of sera 1:2.

Table 4. Neutralization activities against MGI strain of sera taken from patients with tyhroiditis and from healthy people

Neutralization tests with MGI strain and sera from patients with:		Neutralization tests with MGI strain and sera from healthy people[a]
Thyroiditis subacuta de Quervain[a]	Thyreosis[a]	
15 highly positive[b] 5 moderately positive or slightly positive 8 negative	1 moderately positive	13 negative 1 slightly positive 1 moderately positive

[a] Dilution of sera 1:2 or 1:10.
[b] Highly positive: > 75% "plaque" reduction; moderately or slightly positive: 75 to 50% "plaque" reduction; negative: < 50% "plaque" reduction.

Examination of Serum from People who were in Close Contact for a Long Time with Patients Suffering from Subacute Thyroiditis de Quervain

We examined the sera of 3 persons exposed to prolonged contact with patients suffering from thyroiditis de Quervain. All three sera showed significant levels of antibodies against MGI strain. In 2 cases typical thyroiditis developed; the third one had only regional lymphadenitis and subfebrile temperatures. In the first case (Table 5), the contact was a 14-year-old girl whose mother was suffering from

Table 5. Clinical signs and presence of MGI-strain-neutralizing antibodies in sera of persons in close, prolonged contact with patients suffering from thyroiditis subacuta de Quervain

Presumed source of the infection with positive dg. thyroiditis subacuta de Q.	NT with patient convalescent sera	Diagnosis	Presence of MGI-strain-neutralizing antibodies in serum of convalescent patients	Persons in close contact with patient suffering from thyroiditis subacuta
M.G., 34 years Dec. 1972—Jan. 1973[a]	Highly positive	Lymphadenitis cervicalis, subfebrile	Moderately positive	Z.G., 14 years, daughter Jan.—Febr. 1973
F.J., 34 years Dec. 1972—Aug. 1973	n.d.	Subfebrile, Struma diff. eufun., St. post Thyroiditis subacuta?	Highly positive	J.J., 37 years, husband November 1973
J.B., 37 years April 1973—Nov. 1973	Moderately positive	Thyroiditis subacuta de Quervain	Highly positive	J.J., 44 years, colleague Nov. 1973

n.d. = not done.
[a] Period of health disorders.

thyroiditis de Quervain. During the mother's illness the girl repeatedly suffered from lymphadenopathy of the cervical region with subfebrile temperature, the disorders being similar to those of her mother. They appeared 7 months after the first attack of typical thyroiditis (see Table 2). The serum of both patients showed neutralization activities against the MGI strain. The second case was a man, who, a few weeks after his wife had overcome subacute thyroiditis, was hospitalized with status febrilis, expressed struma diffusa eufunctionalis, and had presumably overcome thyroiditis subacuta. Sera of both patients showed MGI strain-neutralizing activity. The third pair of the patients were physicians who came into contact in their common office. The source of infection was a patient with persistent pain in the region of struma, intermittent subfebrile temperature, and weakness. The patient was treated only ambulantly. The contact was the colleague who suddenly developed signs of typical subacute thyroiditis de Quervain. Both patients had high MGI-strain-neutralizing serum activities.

The first 2 patients from Table 5 (M.G. and Z.G.) were further checked serologically for the presence of antibodies against various other infectious agents (Table 6). Besides the positivity of the convalescent sera against the MGI strain of the isolated virus, both sera also showed significant titers of antibodies against the Epstein-Barr virus capsid antigen. Complement fixation activity with cytomegalo virus antigen was not present. The Paul-Bunnel reaction was negative for both cases. 1 patient (M.G) had transitory increased titers against toxoplasma gondii.

Attempts to Identify the Isolated Viruses by Serological, Hemadsorption, and Hemagglutination Tests

As shown on Table 7, neither we nor Dr. H. zur Hausen from the University of Erlangen-Nürnberg (personal communication) were able to show any relationship between the isolated MGI strain and herpes simplex virus 1 and 2, varicella-zoster virus or cytomegalovirus, using complement fixation tests and immunofluorescence. Herpes viruses have been ruled out also in molecular hybridization tests with the isolated MGI strain (zur Hausen, personal communication). The Paul-Bunnel reaction with patient serum was negative, too, though the indirect immunofluorescence test for detection of antibodies against viral capsid antigen of Epstein-Barr virus (EB-VCA) was positive in several sera of tested patients (Table 8).

Neutralization tests with human hyperimmune sera against measles, rubella, mumps, and influenza viruses were also negative. Negative results were also obtained in complement fixation tests with MGI-strain antigen and hyperimmune guinea-pig sera against respiratory syncytial virus (kindly performed by Dr. Bručková and Dr. Syruček at the Institute for Epidemiology and Microbiology in Prague).

In addition, attempts to demonstrate neuramininidase activity in the isolated MGI-strain material were unsuccessful (Dr. Russ, the Institute of Virology, Slovac Academy of Sciences).

Hemadsorption tests on infected human diploid cells treated with 0.5% suspension of rooster red blood cells were negative. Negative results were also obtained with hemagglutination tests using rooster or human-O red blood cells

Table 6. Results of some serological tests with sera of a patient suffering with subacute thyroiditis de Quervain and her serologically positive contact

Patient age	Diagnosis	Samples taken	Serological tests performed:					Notes
			MGI strain[a]	CMV[b]	EB-VCA[c]	Paul-Bunnel	Toxoplasmosis[b]	
M.G., 34 years	Thyroiditis	13.12.72	negative	< 1:2	1:320	< 1:4	1:16	Presumed
	subacuta de Q.	16.1.73	slightly positive	< 1:2	n.d.	n.d.	1:128	source of
		7.6.73	highly positive	< 1:2	n.d.	n.d.	1:16	the infection
		15.11.73	moderately positive	n.d.	1:160	< 1:4	n.d.	of Z.G.
Z.G., 14 years	Lymphadenitis	3.2.73	negative	< 1:2	n.d.	< 1:4	< 1:4	Infection
	cervicalis,	10.2.73	n.d.	n.d.	1:80	n.d.	n.d.	acquired
	subfebrils.	15.11.73	moderately positive	< 1:2	1:160	< 1:4	< 1:4	from M.G.?

n.d. = not done.
[a] Virus neutralization test.
[b] Complement-fixation reaction.
[c] Immunofluorescent test.

Table 7. Some serological results with patient sera and known viral antigens or with MGI strain and known hyperimmune sera

NT with MGI strain and hyperimmune patient sera against[a]:				Complement-fixation reaction with:		Immunofluorescent test		Paul-Bunell reaction
Measles	Rubeola	Influenza/A2	Mumps	RSV[b]	CMV[c]	Varicella-zoster[d]	Herpes simplex[d]	
3 negative	2 negative	2 negative	2 negative	1 negative	4 negative 1 positive	1 negative	2 negative	2 negative

[a] Dilution of all sera tested 1:2.
[b] CFR with MGI strain antigen and hyperimmune guinea pig serum against RSV (respiratory syncytial virus).
[c] CFR with cytomegalo virus antigen and convalescent serum positive against MGI strain.
[d] Indirect immunofluorescent test with MGI strain-infected RLC and hyperimmune sera against either varizella-zoster or herpes simplex viruses.

Table 8. Presence of antibodies against EB-VCA and MGI strain

Patient, age	Titer of anti EB-VCA antibodies[a]	Antibodies against MGI strain	Notes
M.T., 48 years	1:80	positive	convalescent serum
T.K., 25 years	1:40	positive	convalescent serum
H.V., 49 years	1:160	positive	convalescent serum
M.H., 35 years	1:80	positive	convalescent serum
M.G., 34 years	1:320	negative	serum taken in acute stage of infection
M.G., 34 years	1:160	positive	convalescent serum
Z.G., 14 years	1:80	negative	serum taken in acute stage of infection
Z.G., 14 years	1:160	positive	convalescent serum

[a] Highest dilution of antibodies against EB-VCA tested by indirect immunofluorescence.

treated with infectious tissue-culture supernatants or allantoic fluid of 8 to 10-day-old chick embryos infected with an MGI viral agent. The allantoic fluids of MGI-virus-infected chick embryos also were not infectious for RLC cultures.

An accidental infection of RLC cultures by a virus contaminating rabbits or calf serum can be excluded on the following observations: a) positive sero-conversion of the antiviral antibodies in patients with positive isolation of the virus; b) the inability to demonstrate the virus in rabbit-lung cell cultures by blind passages of culture media or by electronmicroscopy; c) positive isolation of the virus from patient materials regardless the type of serum used for tissue cultures.

Discussion

The isolation of 6 viral agents with apparently very similar properties points to a causal relationship between the isolates and subacute thyroiditis de Quervain. The presence of the viruses in the serum and urine of the patients, and particularly in aspiration biopsies of the affected thyroid glands, support this assumption. Isolation of the virus and detection of specific antibodies in acute- and convalescent-patient sera on the one hand, and the low percentage and the low levels of such antibodies in sera of healthy people or patients with other infectious diseases on the other hand, favor a direct etiological relationship between the isolated viral agents and de Quervain thyroiditis.

Since reisolation of the virus several months after the first attack of thyroiditis was successful, it could be assumed that the virus persists in the organism even after clinical symptoms have disappeared. Reinfection, however, cannot be excluded, because the relatively low level of the virus-neutralizing antibodies or, on the other hand, the low affinity of such antibodies to the virus, might be the factor that allows the virus to persist in the organism. Some authors reported thyroid inflammation as lasting 3 to 24 weeks (Bergen Jr., 1958). Repeated attacks of thyroiditis de Quervain are rather common (Janotka *et al.*, 1974). The virus persisting in an individual may not necessarily cause typical thyroiditis de Quer-

vain, as suggested by our observations in which positive isolations of the virus or the presence of significant levels of specific antibodies against MGI virus were accompanied by more or less general lymphadenopathy with subfebrile temperatures but no apparent inflammation of the thyroid gland. A more detailed account of the clinical features of the cases of thyroiditis subacuta de Quervain related to this report will be published elsewhere (Janotka *et al.*, 1974).

The provocative findings in patients suffering from inflammatory affections of the thyroid gland and in their close contacts are in a good agreement with the known fact that de Quervain thyroiditis can sometimes be clinically asymptomatic (Woolner *et al.*, 1962).

Further work is necessary for an exact classification of the isolated viruses. Some morphological, cytological, and further biological features of the isolated viruses will be published later (Stanček *et al.*, submitted for publication; Werner *et al.*, in preparation).

We wish to thank Mrs. E. Schinglerová for her very competent technical assistance.

References

Bergen, S. S., Jr.: Acute nonsuppurative thyroiditis. Arch. intern. Med. **102**, 747—760 (1958)

Eylan, E., Zmucky, R., Sheba, Ch.: Mumps virus and subacute thyroiditis. Evidence of a causal association. Lancet **1957**, 1062—1063

Felix-Davis, D.: Autoimmunisation in subacute thyroiditis. Lancet, **1958**, 880—883

Greene, J. N.: Subacute thyroiditis. Amer. med. J. **51**, 97—108 (1971)

Henle, G., Henle, W., Diehl, V.: Relation of Burkitt's tumor associated herpes-type virus to infectious mononucleosis. Proc. nat. Acad. Sci. (Wash.) **59**, 94 (1968)

Janotka, M., Pecháň, J., Stanček, D., Hnilica, P.: Thyroiditis subacuta de Quervain. Bratisl. lek. Listy **62**, 725—737 (1974)

Maruyama, K., Dmochowski, L., Romero, J. J., Swearingen, G. R.: A virus-containing buffy-coat cell line (Ma-l) derived from a patient with chronic thyroiditis and thyroid carcinoma. Med. Rec. (Houston) **61**, 388—396 (1968)

Stanček, D., Gressnerová, M.: A viral agent isolated from a patient with subacute thyroiditis de Quervain type. Acta virol. **18**, 365 (1974)

Szánto, J.: Stable cell strains from rabbit and rat lung tissue suitable for the propagation of herpes simplex virus. Acta virol. **4**, 380—382 (1960)

Volpé, R., Row, V. V., Ezrin, C.: Circulating viral and thyroid antibodies in subacute thyroiditis. J. clin. Endocr. **27**, 1275—1284 (1967)

Werner, S. C., Ingbar, S. H.: The thyroid, p. 914. New York-London: Harper and Row 1971

Woolner, L. B., Mc Conahey, W. M., Beahrs, O. H.: The surgical aspects of thyroiditis. Amer. J. Surg. **104**, 666—671 (1962)

Daniel Stanček, M. D.
Regional Hygiene Station
Division of Virology
Regional Institute of Health
Trnavská cesta 60
Bratislava, ČSSR