Parietaal cel antistoffen, chronische gastritis en pernicieuze anemie.

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SAMENVATTING

De bevinding, dat parietaal cel antistoffen voorkomen bij patienten met pernicieuze anemie, was het uitgangspunt van de in dit proefschrift beschreven onderzoekingen. Het doel van deze onderzoekingen was een nader inzicht te verkrijgen in de etiologie en pathogenese van pernicieuze anemie.

In het eerste hoofdstuk werd de historische achtergrond van het ziektebeeld pernicieuze anemie belicht en werden de symptomatologie en diagnostiek van deze ziekte in het kort besproken. Er werd een overzicht gegeven van de literatuur over intrinsic factor antistoffen, complement-bindende antistoffen tegen maagslijmvlies en parietaal cel antistoffen. Van deze antistoffen wordt algemeen aangenomen, dat het autoantistoffen zijn.

Intrinsic factor antistoffen komen voor bij ongeveer de helft van de lijders aan pernicieuze anemie; complement-bindende antistoffen en parietaal cel antistoffen respectievelijk bij ruim 60 en bijna 90 procent van de patienten met deze ziekte. De laatstgenoemde antistoffen worden in tegenstelling tot intrinsic factor antistoffen ook aangetroffen bij patienten met schildklierziekten, lijders aan diabetes mellitus en bij patienten met ferriprive anemie en ook, zij het in geringe frequentie, bij normale personen.

Naar aanleiding van de resultaten van een onderzoek verricht door BAUR e.a. (1965) wordt aangenomen, dat complement-bindende antistoffen gericht zijn tegen een niet in water oplosbaar lipoproteine in de wand van de microsomen van de parietale cellen van maagslijmvlies en dat parietaal cel antistoffen identiek zijn met deze complement-bindende antistoffen.

Het tweede hoofdstuk beschrijft de door ons toegepaste methoden van onderzoek.

Parietaal cel antistoffen en complement-bindende antistoffen werden aangetoond door indirecte immunofluorescentie met behulp van antisera geconjugeerd met fluoresceine-isothiocyanaat en gericht tegen respectievelijk menselijk globuline en menselijk complement. Bij bepaalde experimenten werd de directe immunofluorescentie methodiek toegepast alsmede enkele modificaties van de indirecte methode. Antistoffen tegen intrinsic factor werden aangetoond met de dialyse en kool-adsorptie methodieken.

Het tweede deel van het hoofdstuk handelt over de methoden van onderzoek naar de functionele toestand en de structuur van het maagslijmvlies van patienten en naar het gehalte aan vitamine B_{12} in hun serum. De functionele toestand van het maagslijmvlies werd nagegaan door middel van een onderzoek naar de aanwezigheid van „vrij zuur" in het maagsap en met behulp van een onderzoek naar de resorptie van radioactief vitamine B_{12}. De structurele toestand werd onderzocht door middel van histologische beoordeling van maagfundusbiopsieën. Eventueel in het maagslijmvlies aanwezige ontstekingsreacties werden geclassificeerd als: a: superficiele gastritis, b. chronische multifocale gastritis, c. diffuus atrophische gastritis en d. maagatrofie. Het gehalte aan vitamine B_{12} in het serum werd bepaald door middel van de Lactobacillus leichmanii methode.

Het derde hoofdstuk beschrijft de resultaten van experimenten met immunofluorescentie methodieken.

Het resultaat van de indirecte immunofluorescentie methode voor het aantonen van parietaal cel antistoffen berust op een specifieke reactie tussen in de IgG fractie van serum gelocaliseerde antistoffen en in de parietale cellen van maagslijmvlies gelocaliseerd antigeen. De op immunofluorescentie gebaseerde complement-bindingsreactie is afhankelijk van een zich als complement gedragende serumfactor.

In cryostaat coupes van maagfundusbiopsieën van personen met „vrij zuur" in het maagsap en met parietaal cel en complement-bindende antistoffen in het serum kon geen globuline of complement in de parietale cellen worden aangetoond door de coupes te incuberen met een tegen menselijk globuline of complement gericht antiserum. Indien echter het serum van deze personen eerst op de coupes van hun eigen maagslijmvlies werd gebracht en hiermee ge-

incubeerd, kon zowel een binding van globuline als van complement worden aangetoond met de geconjugeerde antisera. Dit wil dus zeggen dat een binding in vivo van parietaal cel en complement-bindende antistoffen, die in vitro reageren met de parietale cellen van eigen maagslijmvlies, niet kon worden waargenomen.

Het immunofluorescentie beeld van parietaal cel antistoffen verschilt van dat van complement-bindende antistoffen. Parietaal cel antistoffen geven een diffuse fluorescentie van het cytoplasma van de parietale cellen, terwijl complement-bindende antistoffen vooral de cel- en kernmembraan van deze cellen doen oplichten. Dit verschil in immunofluorescentie beeld suggereert, dat bij lijders aan pernicieuze anemie antistoffen tegen twee verschillende antigenen van parietale cellen voorkomen. Een andere mogelijkheid is, dat parietaal cel antistoffen en complement-bindende antistoffen tegen éénzelfde antigeen zijn gericht en dat het verschil in immunofluorescentie beeld van deze antistoffen berust op een verschil in de wijze waarop dit antigeen is gelocaliseerd in de cel- en kernmembraan van parietale cellen en in het cytoplasma van deze elementen.

Het vierde hoofdstuk handelt over het voorkomen van parietaal cel antistoffen.

De antistoffen werden gevonden bij 111 van 127 patienten met pernicieuze anemie (87,4 procent) en bij 22 van 754 controle personen (2,9 procent). Verder werden zij aangetroffen bij 44 van 146 patienten met schildklierziekten (30,2 procent) en bij 58 van 486 lijders aan diabetes mellitus (12,1 procent).

Door andere onderzoekers is gevonden, dat patienten met aandoeningen van de schildklier en lijders aan diabetes mellitus relatief vaak chronische gastritis hebben en dat patienten met deze ziekten vaker lijden aan perniceuze anemie dan overeenkomt met de frequentie van voorkomen van deze vorm van anemie in willekeurige bevolkingsgroepen. Indien het voorkomen van parietaal cel antistoffen een wezenlijk kenmerk is van pernicieuze anemie, dan suggereren de bovengenoemde bevindingen, dat het vaak voorkomen van parietaal cel antistoffen bij patienten met bovengenoemde aandoeningen samenhangt met het relatief vaak voorkomen van chronische gastritis en ook van pernicieuze anemie bij deze patienten. Indien deze opvatting juist is, is de relatie tussen het voorkomen van parie-

taal cel antistoffen en chronische gastritis bij patienten met schildklierziekten en bij lijders aan diabetes mellitus duidelijker dan bij patienten met ziekten van de maag, waarvan bekend is, dat zij vaak samengaan met chronische gastritis. Wij vonden namelijk, dat parietaal cel antistoffen niet in verhoogde frequentie voorkomen bij patienten met carcinoma ventriculi, ulcus pepticum ventriculi en bij patienten met klachten ontstaan na partiele maagresectie.

De bovengenoemde overwegingen waren voor ons reden een nader onderzoek te verrichten naar de betekenis van het voorkomen van parietaal cel antistoffen bij niet aan pernicieuze anemie lijdende patienten (hoofdstuk V). Verder verrichtten wij een vergelijkend onderzoek van chronische gastritis mét en zónder parietaal cel antistoffen (hoofdstuk VII).

In het vijfde hoofdstuk zijn de resultaten vermeld van een onderzoek naar de betekenis van het voorkomen van parietaal cel antistoffen bij niet aan pernicieuze anemie lijdende patienten. Nagegaan werd, of parietaal cel antistoffen samen voorkomen met afwijkingen, inherent aan pernicieuze anemie, zoals chronische gastritis, achloorhydrie en een stoornis in de resorptie van vitamine B_{12}.

Het bleek, dat patienten met parietaal cel antistoffen meestal chronische gastritis hebben, vaak achloorhydrie en in een kleiner deel der gevallen een stoornis in de resorptie van vitamine B_{12}. Sommige niet aan pernicieuze anemie lijdende patienten met parietaal cel antistoffen hebben intrinsic factor antistoffen en een laag gehalte aan vitamine B_{12} in het serum.

Het zesde hoofdstuk handelt over een familieonderzoek van pernicieuze anemie.

Het voorkomen van parietaal cel antistoffen werd nagegaan in 54 families van 60 patienten met pernicieuze anemie. De antistoffen waren aanwezig bij 128 van 529 bloedverwanten (24,2 procent) en bij 5 van 114 aangetrouwde familieleden (4,4 procent). De frequentie van voorkomen van parietaal cel antistoffen in een aantal volledig onderzochte gezinnen werd berekend en de wijze van overerving van parietaal cel antistoffen werd onderzocht.

Bij 56 bloedverwanten mét parietaal cel antistoffen werd een onderzoek verricht naar het voorkomen van afwijkingen, inherent aan pernicieuze anemie. Het bleek, dat bloedverwanten met parie-

taal cel antistoffen meestal chronische gastritis hebben, vaak achloorhydrie en in een kleiner deel der gevallen een stoornis in de resorptie van vitamine B_{12}. Bij een aantal van hen werden intrinsic factor antistoffen gevonden, en bij sommigen een laag gehalte aan vitamine B_{12} in het serum.

Het grote verschil in frequentie, waarin parietaal cel antistoffen aanwezig zijn bij verwante en niet verwante familieleden van lijders aan pernicieuze anemie, pleit voor de opvatting, dat het voorkomen van deze antistoffen erfelijk wordt bepaald. Een ander punt, dat hiervoor pleit, is de wijze waarop deze antistoffen worden overgeërfd.

De kenmerken, die het overdrachtspatroon van parietaal cel antistoffen karakteriseren en de frequentie, waarin deze antistoffen in een aantal volledig onderzochte gezinnen werden aangetroffen, zijn sterke argumenten voor de hypothese, dat het voorkomen van parietaal cel antistoffen bij lijders aan pernicieuze anemie en bij een deel van hun verwanten wordt bepaald door een autosomaal dominant gen met verminderde penetrantie.

De hoge frequentie van voorkomen van parietaal cel antistoffen bij lijders aan pernicieuze anemie en het vrijwel altijd aanwezig zijn van afwijkingen, inherent aan deze ziekte, bij bloedverwanten met parietaal cel antistoffen maken het waarschijnlijk, dat de ontwikkeling van pernicieuze anemie in belangrijke mate wordt bepaald door hetzelfde gen, dat verantwoordelijk is voor de aanwezigheid van parietaal cel antistoffen. De verschillende afwijkingen bij pernicieuze anemie, zoals het voorkomen van antistoffen, de chronische gastritis, de achloorhydrie en de stoornis in de resorptie van vitamine B_{12}, kunnen worden opgevat als fenen van één pleiotroop patroon, dat door een autosomaal dominant gen wordt beheerst. De stapsgewijze verwezenlijking van de verschillende fenen geeft verschillende klinische uitingsvormen. De meest volledige uitingsvorm met volle penetrantie van alle fenen is pernicieuze anemie met parietaal cel en intrinsic factor antistoffen. Onvolledig expressie van het gen geeft de verschillende situaties zoals die bij bloedverwanten van lijders aan pernicieuze anemie kunnen worden aangetroffen.

Het zevende hoofdstuk beschrijft een vergelijkend onderzoek van chronische gastritis mét en zónder parietaal cel antistoffen.

De structurele en functionele toestand van het maagslijmvlies van 29 lijders aan pernicieuze anemie werd vergeleken met die van twee groepen patienten met achloorhydrie. De ene groep bestond uit 50 patienten mét, de andere uit 33 patienten zónder parietaal cel antistoffen. Gevonden werd, dat chronische gastritis mét parietaal cel antistoffen zowel structureel als functioneel verschilt van chronische gastritis zónder parietaal cel antistoffen. Chronische gastritis mét antistoffen toont bij histologisch onderzoek het beeld van diffuus atrophische gastritis of maagatrofie. Deze vormen van chronische gastritis werden ook aangetroffen bij lijders aan pernicieuze anemie. Niet aan deze ziekte lijdende personen met achloorhydrie en mét parietaal cel antistoffen hebben in ruim de helft der gevallen een stoornis in de resorptie van vitamine B_{12}. De vorm van chronische gastritis, die niet gepaard gaat met het voorkomen van parietaal cel antistoffen, toont bij histologisch onderzoek veelal het beeld van chronische multifocale gastritis. Patienten zónder antistoffen resorberen vitamine B_{12} in het algemeen in normale hoeveelheden.

De ontwikkeling van chronische gastritis mét parietaal cel antistoffen werd bestudeerd door de structurele en functionele toestand van het maagslijmvlies van 69 personen mét parietaal cel antistoffen onderling te vergelijken en te rangschikken naar de ernst van de eventueel bestaande afwijkingen. Naast de bovengenoemde 50 antistof-positieve personen met achloorhydrie werden 19 personen met parietaal cel antistoffen in het serum en met „vrij zuur" in het maagsap onderzocht. Gevonden werd, dat antistof-positieve personen met „vrij zuur" in het maagsap óf normaal maagslijmvlies óf superficiele gastritis óf diffuus atrophische gastritis hebben. Antistof-positieve personen met achloorhydrie hebben óf diffuus atrophische gastritis óf maagatrofie. De stoornis in de resorptie van vitamine B_{12} bij een deel van de laatstgenoemde personen gaat soms gepaard met een te laag gehalte aan vitamine B_{12} in het serum. Bij een aantal parietaal cel antistof-positieve personen met achloorhydrie werden intrinsic factor antistoffen gevonden. Het bleek, dat het voorkomen van intrinsic factor antistoffen bij deze personen niet altijd samengaat met een stoornis in de resorptie van vitamine B_{12}.

Geconcludeerd werd, dat chronische gastritis met parietaal cel antistoffen in beginsel een progressieve afwijking is. De afwijking is gekenmerkt door een diffuus verlies van parietaal en pepsinogeen

cellen in het maagslijmvlies. Dit leidt eerst tot een verminderde secretie van zoutzuur en later tot een geleidelijk in ernst toenemende stoornis in de productie van intrinsic factor, zodat uiteindelijk pernicieuze anemie kan ontstaan. Uitwendige factoren lijken van invloed te zijn op de ontwikkeling van chronische gastritis met parietaal cel antistoffen tot pernicieuze anemie.

Het voorkomen van parietaal cel antistoffen is, voor zover nu bekend, de vroegst waarneembare afwijking in de ontwikkeling van die vorm van chronische gastritis, die kan leiden tot pernicieuze anemie. Antistoffen tegen intrinsic factor lijken daarentegen pas laat op te treden in de ontwikkeling van chronische gastritis met parietaal cel antistoffen. Er zijn aanwijzingen, dat parietaal cel antistoffen verdwijnen in de latere stadia van de maaglesie van lijders aan pernicieuze anemie.

De praktische betekenis van de resultaten van het in dit hoofdstuk beschreven onderzoek is, dat met het onderzoek naar het voorkomen van parietaal cel antistoffen die vorm van chronische gastritis kan worden opgespoord, die kan leiden tot een stoornis in de productie van intrinsic factor. Bij personen met parietaal cel antistoffen en met achloorhydrie dient men dan ook te onderzoeken, of de secretie van intrinsic factor voldoende is voor een goede resorptie van vitamine B_{12}. Indien dit niet het geval blijkt is het aangewezen deze mensen prophylactisch te behandelen met vitamine B_{12}.

Het achtste hoofdstuk handelt over een onderzoek bij proefdieren. Hierbij werd nagegaan, of door middel van immunisatie chronische gastritis kan worden opgewekt.

Passieve immunisatie van ratten en caviae met de globuline fractie van serum, dat parietaal cel en intrinsic factor antistoffen bevatte, leidde niet tot het ontstaan van chronische gastritis. Evenmin kon worden angetoond dat de intraveneus ingespoten parietaal cel antistoffen zich in vivo hadden gehecht aan het corresponderend antigeen.

Ook actieve immunisatie van konijnen met maagslijmvlies en maagsap leidde niet tot chronische gastritis. In het serum van met maagslijmvlies behandelde konijnen konden geen parietaal cel antistoffen worden aangetoond.

Het maagslijmvlies van een met maagsap geïmmuniseerde hond

toonde histologische afwijkingen in de zin van chronische gastritis. Helaas werd bij deze hond geen maagfundusbiopsie verricht vóór de immunisatie. Bij twee andere, op dezelfde wijze behandelde honden, werden geen afwijkingen van het maagslijmvlies waargenomen. Bij de hond met chronische gastritis en bij de twee honden zonder deze afwijking werden circulerende antistoffen tegen intrinsic factor aangetoond.

Het negende hoofdstuk handelt over enkele aspecten van de etiologie en pathogenese van pernicieuze anemie.

De suggestie van TAYLOR in 1959, dat pernicieuze anemie een autoimmuun ziekte is, bleek een vruchtbare werkhypothese bij de in de laatste jaren verrichte onderzoekingen naar de pathogenese van pernicieuze anemie. Gevonden werd, dat bij lijders aan pernicieuze anemie antistoffen tegen intrinsic factor en tegen de parietale cellen van maagslijmvlies voorkomen en dat het voorkomen van laatstgenoemde antistoffen bij personen zonder verschijnselen van gebrek aan vitamine B_{12} gepaard gaat met het bestaan van een specifieke vorm van chronische gastritis, die kan leiden tot pernicieuze anemie. Deze bevindingen en het feit, dat intrinsic factor antistoffen op experimentele wijze kunnen worden opgewekt bij proefdieren, steunen de hypothese van TAYLOR, dat een autoallergisch proces een rol speelt in de ontwikkeling van pernicieuze anemie.

Dat een autoallergische reactie van intrinsic factor antistoffen een belangrijke rol speelt in de ontwikkeling van pernicieuze anemie bij patienten met een reeds door chronische gastritis verminderde productie van intrinsic factor, lijkt niet waarschijnlijk. Ook zijn er ons inziens geen redenen om aan te nemen, dat deze antistoffen en/of parietaal cel antistoffen op zich verantwoordelijk zijn voor de ontwikkeling van die vorm van chronische gastritis, die kan leiden tot een stoornis in de productie van intrinsic factor. Wij geven de voorkeur aan de hypothese dat de vorming van parietaal cel antistoffen en van intrinsic factor antistoffen secundair is aan degeneratieve afwijkingen van het maagslijmvlies van personen met die vorm van chronische gastritis, die kan leiden tot pernicieuze anemie. Indien men de situatie bij pernicieuze anemie mag vergelijken met die bij de ziekte van Hashimoto, is het waarschijnlijk, dat een autoallergisch proces door een cellulair mechanisme van pathogenetische betekenis is in de ontwikkeling van pernicieuze anemie.

SUMMARY

The investigations, which are described in this thesis were initiated after the discovery of the autoantibodies against gastric parietal cells in 1962.*

The diagnostic significance of these parietal cell antibodies was investigated in a series of clinical and laboratory studies performed in a number of antibody-positive subjects with or without established pernicious anemia.

Special attention was paid to the incidence of parietal cell antibodies in relatives of pernicious anemia patients. This made it possible to analyse the family segregation of both parietal cell antibodies and pernicious anemia.

Some experiments using passive and active immunization were performed in laboratory animals with the purpose of obtaining an experimental model for the study of pernicious anemia.

In the final chapter the significance of the recently acquired clinical, genetic and experimental data for the pathogenesis of pernicious anemia is discussed.

Chapter one deals with the historical development of our knowledge of pernicious anemia. In the present study this term is used only when the patient showed clinical signs and symptoms of vitamin B_{12} deficiency due to a failure of the stomach to secrete sufficient amounts of Castle's intrinsic factor. The impaired production of intrinsic factor is caused by a chronic inflammatory disorder of the gastric mucosa which at present is regarded as the primary disorder in pernicious anemia.

In recent years it has been shown that autoantibodies against constituents of the gastric mucosa occur in association with pernicious anemia. Initially the presence of an inhibitor of intrinsic factor was

* References are given at the end of each chapter.

demonstrated by SCHWARTZ (1958) and by TAYLOR (1959) in the sera of some patients by means of an in vivo test. The subsequent development of in vitro methods for the detection of intrinsic factor antibodies revealed that two different antibodies can be distinguished. One of these has affinity for the binding site of vitamin B_{12} to intrinsic factor (ABELS et al., 1963 b), while the other reacts with intrinsic factor which has bound vitamin B_{12} (JEFFRIES et al., 1962). About 50 percent of the patients with pernicious anemia have intrinsic factor antibodies.

A second approach to the demonstration of autoantibodies in pernicious anemia has been made by complement-fixation tests (IRVINE et al., 1962; MARKSON and MOORE, 1962) and by indirect immunofluorescent techniques (IRVINE, 1963; TAYLOR et al., 1962; ABELS et al., 1963 c). Complement-fixing antibodies reactive with extracts of gastric mucosa occur in about 60 percent of patients with pernicious anemia while parietal cell antibodies may be detected by the immunofluorescent method in more than 80 percent. Both the complement-fixing and the parietal cell antibodies have been found in a small proportion of apparently healthy control subjects. The incidence of these antibodies is increased in Hashimoto's disease and in primary hypothyroidism, in thyrotoxicosis, iron deficiency anemia and in diabetes mellitus. It has been proposed by BAUR et al. (1965) and by TAYLOR et al. (1962) that the complement-fixing antibodies are identical with the parietal cell antibodies.

In chapter two the technical aspects of this study are described.

The first part of this chapter deals with the immunological methods for the detection of gastric autoantibodies.

1. Parietal cell antibodies were demonstrated by means of the indirect immunofluorescence technique of Coons and Kaplan using rat gastric fundus mucosa as the antigen substrate and fluoresceïn-conjugated rabbit-antihuman globulin-globulin as the conjugate. In some experiments the indirect method was modified and the direct immunofluorescence test was used (see chapter III).
2. Complement-fixing gastric antibodies were demonstrated by an indirect immunofluorescent complement staining technique using human gastric fundus mucosa as the substrate and fluoresceïn-labeled rabbit antiserum to human complement as the conjugate.

3. Intrinsic factor antibodies, reactive with the binding site of vitamin B_{12} of this factor, were demonstrated in the patients sera by the charcoal-absorption test and in the sera of laboratory animals by the dialysis method.

The second part of chapter II describes the studies performed in patients.

1. Function tests of gastric secretory status: a. The hydrochloric acid output of the gastric mucosa was studied by aspiration of gastric juice during two hours after stimulation of the secretion with 0.5 mg histamine diphosphate given by subcutaneous injection and by determining the acid content of the juice with 0.1 n NaOH using Töpfer's reagent as an indicator. Only the presence or the absence of "free acid" in gastric juice are reported in this study. b. In a number of patients the hydrochloric acid secretory capacity of the gastric mucosa was investigated by the diagnex blue test. c. Vitamin B_{12} absorption was investigated as a parameter of intrinsic factor production with Schilling's urinary excretion test. The normal values for this test vary in our laboratory from 15 to 46 percent, while the average is 29 percent.

2. Histological investigation of the gastric mucosa. Gastric biopsies were obtained under fluoroscopic control with the capsule of Crosby and Kugler from the body area of the stomach. For histological examination the biopsy material was fixed in 10 percent formol-saline and embedded in paraffin wax. Pairs of sections were stained with hematoxylin-eosin and with the periodic-acid-Schiff reaction. The sections were examined independently by two persons. Chronic inflammatory changes of the gastric mucosa were classified as: a. superficial gastritis (figure II-2, p. 22), b. chronic multifocal gastritis (figure II-3, p. 23), c. diffuse atrophic gastritis (figure II-4, p. 24) and d. gastric atrophy (figure II-5, p. 25).

3. The serum vitamin B_{12} content was estimated by means of the Lactobacillus leichmanii method. In our experience a vitamin B_{12} level lower than 0.1 ng per ml serum is abnormal. Levels between 0.1 and 0.2 ng vitamin B_{12} per ml serum may be considered as moderately decreased.

Chapter three deals with the results of some experiments using immunofluorescent methods.

Part A gives the results of the investigations about the specificity of the methods for the detection of parietal cell antibodies and of complement-fixing antibodies.

1. Parietal cell antibodies are demonstrable by direct incubation of gastric mucosal sections with fluoresceïn-conjugated testsera.
2. Indirect immunofluorescent staining of the gastric parietal cells with conjugated rabbit antiserum to human complement is dependent on a complement-like serum factor (table III-1, p. 29). The indirect staining reaction with conjugated rabbit antiserum to human globulin depends on a serum factor that reacts with an anti-human IgG globulin in the conjugate (table III-2, p. 30).
3. Immunofluorescence due to staining of parietal cell antibodies and of complement-fixing antibodies is inhibited by preincubation of the sections with non-conjugated antisera to human globulin and to human complement (table III-3, p. 31).
4. Immunofluorescence due to parietal cell antibody uptake is abolished by absorption of antibody-positive testsera with extracts of gastric fundus mucosa of human and rat origin (table III-4, p. 33).

Part B deals with the results of some experiments about the nature of the parietal cell antibodies and about the in vivo reactivity of these antibodies and of the complement-fixing gastric antibodies.

1. Immunofluorescent detection of parietal cell antibodies with fluoresceïnated antisera to human IgG, IgA and IgM showed these antibodies in the IgG fraction of 6 antibody-positive sera (table III-5, p. 34).
2. Exposure of sections of gastric mucosal specimens, obtained from 5 parietal cell antibody-positive subjects with hydrochloric acid in their gastric juice, to conjugated antisera against human IgG and human complement produced fluorescence of some mononuclear cells in the lamina propria of the substrates, but failed to stain the parietal and other gastric cells. When the substrate had been exposed to the patient's own serum before the incubation with the fluorescent antisera, deposition of globulin and complement was observed in the parietal cells. Staining with fluorescent antiglobulin serum gives a coarsely granular fluorescence of the entire cytoplasm of the parietal cells (figure III-1, p. 35). The application of anticomplement serum results in a fluorescence especially of the cellular and nuclear membranes of the parietal cells and only in a faint diffuse

fluorescence of the cytoplasm of these elements (figure III-2, p. 36). Heating the autologous sera to 56° C. for 30 minutes as well as dilution in Na-EDTA abolishes the staining due to complement-fixing gastric antibodies (figure III-3, p. 37).

Part C of this chapter gives data about the incidence of complement-fixing gastric antibodies in 39 pernicious anemia patients. The antibodies were detected in 23 out of 26 patients with and in 1 out of 13 patients without parietal cell antibodies in their serum (table III-6 and III-7, p. 39).

The results presented in this chapter clearly indicate that true autoantibodies are demonstrated by the immunofluorescent techniques for the detection of parietal cell antibodies and of complement-fixing gastric antibodies. Involvement in vivo of these autoantibodies which react in vitro with the individual's own parietal cells could not be demonstrated. Parietal cell antibodies, which are present in the IgG fraction of serum, react with a granular cytoplasmic component of the gastric parietal cells while complement-fixing antibodies seem to have affinity especially for the cellular and nuclear membranes of these elements. This observation suggests the possibility that gastric autoantibodies exist, which react with two different antigenic components of the gastric parietal cells. An alternative explanation is that the different fluorescence patterns observed with fluoresceïnated anti IgG and anticomplement sera depend on a steric difference of identical antigenic sites which influences the uptake of complement.

Chapter four is a report of the results of our study about the incidence of parietal cell antibodies.

As shown in table IV-1 (p. 43) parietal cell antibodies were detected in 111 out of 127 pernicious anemia patients (87.4 percent) and in 22 out of 754 normal controls (2.9 percent). A correlation between the incidence of the antibodies and the age or sex of the patients with pernicious anemia or with the duration of the disease could not be established (table IV-2, p. 43). A similar lack of correlation was observed between the incidence of antibodies and the age or sex of the normal control individuals (table IV-3, p. 44). In patients with thyroid disorders and in patients suffering from diabetes mellitus the gastric parietal cell antibodies were detected significantly more often than in normal controls (table IV-4, p. 45).

The serological relationship between pernicious anemia on the one side and thyroid diseases and diabetes mellitus on the other side is in accordance with the well known clinical association between these disorders. Chronic gastritis, which is an inherent feature of pernicious anemia, is also frequently found in association with diseases of the thyroid gland and with diabetes mellitus (table IV-5, p. 46). This suggests an association between the occurrence of parietal cell antibodies and the presence of chronic gastritis. However the incidence of parietal cell antibodies in patients with thyroid disorders and in patients suffering from diabetes mellitus seems to be less than that of chronic gastritis. An even more pronounced discrepancy between the incidence of parietal cell antibodies and that of chronic gastritis appears to be present in patients with gastric cancer, in patients with gastric ulcer and in patients who had been subjected to partial gastrectomy. Though chronic gastritis is known to occur frequently in these groups of patients, only a minority of them were found to have antibodies to gastric parietal cells (table IV-1, p. 43).

The data reported in this chapter prompted us to study the significance of the occurrence of parietal cell antibodies in patients without pernicious anemia (chapter V) and to perform a comparative study of chronic gastritis with and without gastric autoantibodies (chapter VII).

In chapter five an investigation about the significance of the occurrence of parietal cell antibodies in patients without pernicious anemia is reported.

We have studied 33 patients, who were suffering from a variety of disorders other than pernicious anemia and who were, to the best of our knowledge, not related to patients with this disease. The data obtained in these 33 patients are recorded in table V-1 (p. 51). It can be seen from table V-2 (p. 52), that the absence of hydrochloric acid in the gastric juice of 25 of these patients shows a good, but not perfect correlation with the severity of the histological changes and with the intrinsic factor secretory capacity of their gastric mucosa. The presence of antibodies to intrinsic factor, which were detected in 5 out of the 33 patients, was associated with severe structural changes of the gastric mucosa but not necessarily with an impairment of vitamin B_{12} absorption (table V-3, p. 52). In the sera of 3 out of 12 patients in whom the serum vitamin B_{12} activity was estimated subnormal levels were found (table V-4, p. 53).

These results demonstrate that the presence of gastric parietal cell antibodies is nearly always associated with chronic gastritis and frequently with a decreased hydrochloric acid secretion and intrinsic factor production. However the severity of the gastric lesion as judged bij histologic study or the degree of reduction in the gastric secretory functions can not be predicted from the presence of parietal cell antibodies in patients without pernicious anemia

Chapter six is a report of a family study of pernicious anemia. The incidence of parietal cell antibodies was determined in the relatives of 60 pernicious anemia patients. These antibodies were found in 128 out of 529 blood relatives (table VI-1, p. 58) and in five out of 114 allied relatives who were studied as a control group. In order to obtain information about the significance of parietal cell antibodies as a feature of pernicious anemia we investigated 56 antibody-positive relatives for the presence of features inherent to this disease. The results of these investigations are recorded in table VI-5 (p. 64) and are summarized in table VI-6 (p. 65).

The high incidence of parietal cell antibodies in patients suffering from pernicious anemia and the occurrence of features inherent to this disease in the antibody-positive relatives indicate, that the occurrence of parietal cell antibodies is a characteristic of pernicious anemia which may be compared with other essential features of this disease.

The higher frequency of parietal cell antibodies in the blood relatives than in the allied relatives of pernicious anemia patients, in a proportion of roughly 10 : 1, indicates that the presence of parietal cell antibodies may be determined by a genetic factor. Furthermore, we found the antibodies in 41 out of 123 children of 42 antibody-positive individuals whose partners were antibody-negative, i.e. in a frequency of 33.3 percent ($p_1 = 26.81$ percent; $p_2 = 40.56$ percent; $\alpha = 5$ percent). Another argument for this conception is the mode of transmission of the antibodies through the different generations of our families. It was found to be characterized, as illustrated bij a choice out of our pedigrees, by the following features:

1. The occurrence of parietal cell antibodies can be traced through two and more generations (figure VI-3, p. 62).
2. In mixed sibships the antibodies were never found in the offspring

of the negative siblings; however they could be demonstrated in part of the offspring of the positive siblings (figure VI-5, p. 63). 3. When both parents have parietal cell antibodies, part of their children may be negative (figure VI-6, p. 64).

These characteristics are in favour of the hypothesis, that the development of parietal cell antibodies is controlled by one heterozygote dominant gene. Another argument for this hypothesis is, that the alternative possibility of recessive heredity seems positively unlikely, for in six families with two positive parents, only 5 out of 19 children were found to have the antibodies. Furthermore in the case of recessive heredity one had to postulate an unusually high number of heterozygotes in the population to account for the antibody-positive offspring in families with only one positive parent.

Our data are not in favor of a sex linked heredity of the anomaly. A direct argument against this possibility is that we found in our material 5 families in which a positive father and a negative mother had a positive son. In another family the antibodies were found in a father, son and grandson (figure VI-2, p. 60). Altogether the segregation pattern of the anomaly corresponds fairly well to that of a dominant autosomal gene. Seen from the angle of physiologic genetics the various manifestations of pernicious anemia, such as the presence of parietal cell antibodies, the gastric achlorhydria and the impairment of vitamin B_{12} absorption may be regarded as phenes of one pleiotropic pattern, the development of which is controlled by the same dominant autosomal gene.

The penetrance of this gene is, with regard to the production of parietal cell antibodies, certainly lowered, for no antibodies were detected in 16 out of 127 pernicious anemia patients (chapter IV, p. 114). Complete loss of antigenic compounds from the gastric mucosa seems to be responsible for the absence of parietal cell antibodies in a number of pernicious anemia patients (chapter IX ,p. 124). Other factors may be responsible for the discrepancy between the calculated (33.3 percent) and expected (50 percent) frequency of the antibodies in their relatives. That age is a causative factor seems unlikely, for there was no significant difference between the average ages of the parietal cell antibody-positive and negative relatives, which were used for a maximum likelihood estimation (table VI-4, p. 62). The slightly higher frequency of parietal cell antibodies in

the female sex is probably due to a sex-controlled difference in the penetrance of this gene.

Before we may accept the hypothesis that one gene with incomplete penetrance controls the development of pernicious anemia, we have to consider the possibility of a multifactorial heredity of this disease.

It has been suggested by COGHILL et al. (1965) and by FISCHER and TAYLOR (1965) that antibodies to intrinsic factor can play a role in the development of pernicious anemia. One may suppose that the formation of parietal cell antibodies and the development of the gastric lesion in individuals with these antibodies are dependent on the effect of one gene and that another gene controls the formation of intrinsic factor antibodies.

In this case intrinsic factor antibodies would occur with equal frequency in parietal cell antibody-positive relatives of pernicious anemia patients and in those without these antibodies. In one of our investigations we could not detect intrinsic factor antibodies in 50 parietal cell antibody-negative relatives of pernicious anemia patients.

Another possibility is that more than two genetic factors may determine the development of pernicious anemia. An additive multifactorial determination of pernicious anemia, as in the case of intelligence and bodygrowth in man, is excluded by the absence of a quasi-continuous variability of the manifestations in this disease. The hypothesis of a multifactorial control of pernicious anemia can only be acceptable, if one assumes that by the presence of a number of genes with similar additive activities in the genotype a functional threshold is reached, whereby the development of the syndrome and its discontinuous distribution in the population and families is made possible. In this case one should expect that a fair number of antibody-positive cases would descend from antibody-negative parents. In our material the pathway of the affection shows however a striking continuity through the generations. In fact almost all antibody-positive cases whose parents could be investigated had at least one positive parent. Therefore, even if one assumes a genetically controlled threshold reaction, the hypothesis of a plain multifactorial inheritance of pernicious anemia does not appear to be satisfactory.

In our opinion it is much more probable that pernicious anemia

is controlled by the cumulative effects of one major dominant autosomal gene and a number of both external and genetic factors.

Chapter seven deals with a comparative study of chronic gastritis with and without parietal cell antibodies.

Chronic gastritis was studied from the morphological and functional point of view in 131 subjects in whom there were reasons to suspect the presence of this disorder. Among these 131 individuals were: A) Twenty nine patients with pernicious anemia. In this group we included seventeen patients with and twelve without parietal cell antibodies in their serum. B) Sixty nine subjects who had parietal cell antibodies in their serum, but did not have clinical manifestations of vitamin B_{12} deficiency. Among them there were 19 subjects with and 50 without hydrochloric acid in their gastric juice. Thirty six of these 69 parietal cell antibody-positive individuals were related to patients suffering from pernicious anemia (table VI-7, p. 66). The other 33 were selected from the antibody-positive individuals out of a large number of hospital patients (table V-2, p. 52). C) Thirty three achlorhydric subjects without parietal cell antibodies in their serum who were suffering from a variety of disorders (table VII-1, p. 76).

The histological appearance and the intrinsic factor secretory capacity of the gastric mucosa of the achlorhydric subjects with and without parietal cell antibodies were studied and compared with those of the pernicious anemia patients (table VII-2, p. 79). In addition we compared the structural and functional state of the gastric mucosa of the achlorhydric subjects with parietal cell antibodies with that of the antibody-positive subjects who had hydrochloric acid in their gastric juice (table VII-3, p. 79).

The results obtained in the achlorhydric subjects without pernicious anemia indicate that chronic gastritis associated with gastric autoantibodies differs both morphologically and functionally from chronic gastritis without antibodies to parietal cell components.

The chronic gastritis in achlorhydric patients without parietal cell antibodies is usually characterized at histological examination by a focal distribution; diseased areas are found next to areas with intact glandular epithelium. Despite the impairment of hydrochloric acid secretion most of these subjects are able to absorb a normal amount of vitamin B_{12}.

Antibody-positive individuals with achlorhydria however have a type of chronic gastritis which at histological examination cannot be distinguished from the gastric lesion of pernicious anemia patients. In both conditions there is a more or less severe diffuse loss of the specific glandular elements from the gastric mucosa which also shows varying degrees of infiltration by mononuclear cells. In addition a significant number of achlorhydric subjects with parietal cell antibodies have the same degree of impairment of intrinsic factor secretion as found in pernicious anemia. Antibody-positive individuals with hydrochloric acid in their gastric juice have a less advanced degree of damage of their gastric fundus glands than achlorhydric subjects with these antibodies. This difference corresponds with the intrinsic factor secretory capacity of their gastric mucosa for antibody-positive subjects with hydrochloric acid in their gastric juice have a normal Schilling test.

When the various situations occurring within the group of parietal cell antibody-positive individuals are arranged according to the degree of the morphological and functional changes of their gastric mucosa, a clear gradient can be observed from functionally intact fundus glands without detectable structural changes to complete atrophy of these glands associated with loss of hydrochloric acid and intrinsic factor secretion. In addition antibodies to intrinsic factor and low serum vitamin B_{12} values were demonstrated in a number of achlorhydric subjects with parietal cell antibodies (table VII-6 and table VII-7, p. 82).

This gradient of structural and functional gastric defects suggests that the various situations found in parietal cell antibody-positive subjects must be considered as instances of mutually related manifestations of the same basic disorder which also determines the symptomatology of pernicious anemia. Another point in favour of this conception is that we know that there is a common genetic background for the familial occurrence of parietal cell antibodies and pernicious anemia (chapter VI).

On the basis of this evidence we think that parietal cell antibody-positive individuals have a particular type of chronic gastritis which interferes with hydrochloric acid secretion and intrinsic factor production and which therefore may terminate in the development of pernicious anemia. It may be assumed that the progression of chro-

nic gastritis with parietal cell antibodies to pernicious anemia also depends upon a number of exogenous factors.

Apparently parietal cell antibodies already occur in the very early stages of the type of chronic gastritis which may terminate in pernicious anemia. Antibodies to intrinsic factor seem to appear rather late in the development of this disorder, for these antibodies were not detected in parietal cell antibody-positive individuals with hydrochloric acid in their gastric juice and in only 13 out of 50 parietal cell antibody-positive subjects with achlorhydria (table VII-6, p. 82). Our data about the incidence of parietal cell antibodies in pernicious anemia suggest that these antibodies may disappear in patients with a gastric lesion in the stage of complete atrophy.

Because parietal cell antibodies seem to be an early and frequent symptom pointing to the possible development of pernicious anemia, the method for the detection of these antibodies should be employed as a routine procedure in all conditions frequently associated with parietal cell antibodies. Antibody-positive individuals should be investigated for the presence of hydrochloric acid in their gastric juice and when they are found to be achlorhydric, the intrinsic factor secretory capacity of their gastric mucosa should be examined. If they lack intrinsic factor, they should receive prophylactic treatment with vitamin B_{12} before a deficiency of this vitamin leads to irreversible neurologic complications.

In chapter eight the results of some investigations about passive and active immunization in laboratory animals are reported.

Passive immunization was performed by intravenous injection of gamma globulin fractions obtained from pernicious anemia sera in rats and guinea pigs. The procedure failed to evoke inflammatory changes in their gastric mucosa. In cryostat sections of gastric fundus mucosa of these animals we could not detect deposition of antibodies in the parietal cells with the aid of fluorescent antiserum to human globulin. Therefore this attemp to demonstrate an in vivo effect and localisation in parietal cells of parietal cell antibodies, which are known to react in vitro with the gastric parietal cells of rats and guinea pigs, was not succesful.

The investigations about active immunization were performed by means of intramuscular and intracutaneous injection of a suspen-

sion of antigen in Freund's adjuvant in nine rabbits and in three dogs. Six rabbits were sensitized with extracts of gastric mucosa; three additional rabbits received normal human gastric juice. After immunization no structural changes were observed in the gastric mucosal membrane of the rabbits. In the serum of these animals we could not detect antibodies to the gastric parietal cells by indirect immunofluorescent studies with conjugated antiserum to rabbit globulin. After immunization of the dogs with normal human gastric juice a chronic inflammatory lesion of the gastric mucosa was observed in only one of them. The gastric mucosal membrane of this dog was not studied before immunization. Antibodies to intrinsic factor, as demonstrable by the dialysis method, were present in the serum of the dog with chronic gastritis as well as in the sera of the two dogs without a gastric lesion.

In chapter nine some aspects of the etiology and pathogenesis of pernicious anemia are discussed.

In 1959 it has been suggested by TAYLOR that an autoimmune process is involved in the pathogenesis of pernicious anemia. This hypothesis has led to a number of investigations which have demonstrated that about 50 percent of patients with pernicious anemia have circulating antibodies to intrinsic factor and that more than 80 percent of these patients have antibodies against one or more other components of the gastric parietal cells. Parietal cell antibodies have also been detected in a number of apparently normal control subjects in association with a specific type of chronic gastritis which may terminate in pernicious anemia.

It has been stated by WITEBSKY (1958) that the demonstration of autoantibodies in itself is not sufficient evidence to establish autoimmunity as an important pathogenic factor. The same antibodies also should be produced in laboratory animals and in these animals active immunization should induce pathologic changes that resemble the organ lesion in the human disease.

The available data about the incidence of gastric autoantibodies indicate that loss of tolerance to gastric mucosal components may occur in the early stages of the pathogenesis of pernicious anemia. It is however not yet established if in addition to these circulating autoantibodies an autoallergic reaction by a cellular mechanism

occurs even though it is attractive to assume such a process in view of the relationship and the similarity of chronic gastritis and autoimmune thyroiditis.

According to one currently held view antibodies to intrinsic factor may reduce the available amount of intrinsic factor by inactivation in the intestine or at its site of production in the gastric mucosa. In this case these antibodies are considered to be of pathogenic significance in the development of pernicious anemia in patients with chronic gastritis whose production of intrinsic factor is already reduced.

In our opinion the absence of intrinsic factor antibodies in nearly 50 percent of pernicious anemia patients and the detection of these antibodies in a number of parietal cell antibody-positive individuals without a significant impairment of vitamin B_{12} absorption are strong arguments against the hypothesis that these antibodies do play an important role in the pathogenesis of pernicious anemia. Another argument against this hypothesis is the finding of ABELS and coworkers (1963) that the defective absorption of vitamin B_{12} in pernicious anemia patients with intrinsic factor antibodies improves as much as in those patients without these antibodies, when vitamin B_{12} is given simultaneously with normal human gastric juice. Furthermore it has been demonstrated by ARDEMAN and CHANARIN (1965) that the improvement in the absorption of vitamin B_{12} in corticosteroid treated patients with pernicious anemia may occur before a decline in titer of intrinsic factor antibodies can be noted and that there is no difference between the effectiveness of these steroids in improving vitamin B_{12} absorption in patients with and without these antibodies.

The possibility, that antibodies against parietal cell components may have a damaging effect upon the gastric mucosa also seems unlikely, for pernicious anemia may occur in association with hypogammaglobulinemia. In this connection our observations on parietal cell antibody-positive individuals with hydrochloric acid in their gastric juice should also be mentioned. In cryostat sections of gastric mucosal material obtained from these subjects we could not detect deposition of antibody or complement in the parietal cells with the aid of fluorescent rabbit antisera to human globulin and human complement. If however serum from these individuals was applied

to sections of their own fundus mucosa an affinity of globulin to components of the parietal cells as well as fixation of complement by these elements could be visualized. Therefore involvement in vivo of gastric autoantibodies which react in vitro with the individuals own parietal cells could not be demonstrated. An additional argument against a primary pathogenic significance of gastric autoantibodies may be found in our studies on laboratory animals injected with varying amounts of serum gamma globulin obtained from pernicious anemia patients. A damaging effect of these antibodies on the gastric parietal cells and an in vivo uptake by these elements were not observed.

On the basis of the evidence presented above it seems to us more probable that the occurrence of gastric autoantibodies is a secondary phenomenon due to exposure of the antibody producing system to antigenic compounds released from the gastric mucosa of individuals with the particular type of chronic gastritis which may terminate in pernicious anemia. Parietal cell antibodies already occur in a very early stage of development of this type of chronic gastritis. Our data about the incidence of parietal cell antibodies in pernicious anemia patients suggest that these antibodies may disappear in those patients with a gastric lesion in the stage of gastric atrophy, probably as a result of complete loss of antigenic compounds from the gastric mucosa. The late development of intrinsic factor antibodies in parietal cell antibody-positive individuals without clinical signs of vitamin B_{12} deficiency and the absence of these antibodies in nearly 50 percent of pernicious anemia patients may be due to a lower degree of antigenicity of intrinsic factor as compared with that of the antigen of antigens responsible for the development of parietal cell antibodies.

Because a direct pathogenic effect of circulating gastric autoantibodies seems to be at most of minor importance, one is inclined to compare the situation observed in pernicious anemia with that in Hashimoto's disease, in which disease a cellular type of autoallergy is believed to be of greater pathogenic significance than an autoallergic reaction by circulating autoantibodies.

The serological relationship between pernicious anemia, Hashimoto's disease, primary myxedema, hyperthyroidism and idiopathic adrenal insufficiency suggests an etiological relationship between these

disorders. Although there is no direct evidence available at present that diabetes mellitus also belongs to the group of these so-called "idiopathic autoimmune diseases", it is possible that in the future the symptomatic entity of diabetes mellitus will be considered as being composed of different etiological entities, one of which may have a close etiological relationship to pernicious anemia, for parietal cell antibodies occur in about 12 percent of patients with diabetes mellitus.

There are good indications for a genetic control of at least several of the disorders mentioned above. It may be possible that the primary defect in each of them depends on the effect of a different gene. In this case a comparable or very similar peripheral effect of each of these genes may be responsible for the overlapping of the serological abnormalities. Another possibility is that we have to deal with a complex of genetic factors, among which several major genes are present which determine the specific target organ of the symptoms. The third possibility is that one single gene controls to a major extent the development of all the disorders mentioned above. If a cellular immunological process is indeed of pathogenic significance, the primary effect of this gene can be localized either in analogous structures of stomach, thyroid, adrenal and pancreas gland cells or in the immunological apparatus. In the first case it is probably a biochemical defect, occurring late in fetal development of the individual or even after birth, which gives rise to the production of an antigen that is regarded by the immunological competent cells as "not-self". The other possibility is that the primary abnormality is a defect in the part of the immunological apparatus responsible for cellular immunity. Because of this defect naturally occurring antigens are not recognized as "self" by the lymphocytes.

The number of diseases that demonstrate both a clinical and serological relationship is steadily growing. Each of these so called "idiopathic autoimmune diseases" can at present be characterized clinically and genetically in a more or less satisfactory manner. However our knowledge of the biochemical background of these diseases is at present too incomplete to allow a comprehensive etiological synthesis of the different manifestations or on the other hand to point out essential etiological differences between the different clinical syndromes.