BINDING OF RADIOGRAPHIC CONTRAST MEDIA TO SERUM PROTEINS

A clinical and experimental investigation of their adverse effects through influence on active steroid hormone levels

Akademisk avhandling som med vederbörligt tillstånd av rektorsämbetet vid Umeå Universitet för avläggande av medicine doktorsexamen kommer att offentligen försvaras onsdagen den 26 maj 1982, kl 13.15, hörsal D, Samhällsvetarhuset, Umeå Universitet

av

STAFFAN WIrell
med.lic.

Umeå 1982
ABSTRACT

BINDING OF RADIOGRAPHIC CONTRAST MEDIA TO SERUM PROTEINS
A clinical and experimental investigation of their adverse effects through influence on active steroid hormone levels.

by Staffan Wirell, M.D., Department of Diagnostic Radiology, University Hospital of Umeå, S-901 85 Umeå, Sweden.

Summary of thesis based on the following papers:

I Wirell S.: Adverse reactions at intravenous cholegraphy with ioglycamide. Correlation with the level of circulating sex hormones. Accepted for publication in Acta Radiol. Diagn. (1982)


In a clinical study, Paper I, the highest incidence of adverse reactions at intravenous cholegraphy was recorded in young adults; especially in the luteal phase of the menstrual cycle.

In Papers II, III and IV, the interplay between the protein binding of a bile-excreted contrast compound, ioglycamide, and steroid sex hormones was studied. Altered binding characteristics of the steroid hormone-protein binding indicated the possibility of a release of steroid hormones in the presence of ioglycamide. The significance of this finding was discussed in terms of clinical relevance.

In Paper V, the binding of a non-ionic contrast compound to human serum proteins was studied with two different techniques. No, or only little, binding of metrizamide to human serum proteins was demonstrated.

Key words: adverse reactions, ioglycamide, metrizamide, protein binding radiographic contrast media, separation techniques, serum proteins, steroid sex hormone levels
BINDING OF
RADIOGRAPHIC CONTRAST MEDIA
TO SERUM PROTEINS

A clinical and experimental investigation of their adverse effects
trough influence on active steroid hormone levels

by
Staffan Wirell

Umeå 1982
Parenterally administered radiographic contrast media can cause adverse reactions. The highest frequency of severe adverse reactions is seen in connection with intravenous injection of cholegraphic contrast compounds, which are also known to bind to serum proteins. In Paper I, clinical observations of the frequency of adverse reactions, in connection with intravenous cholecography with ioglycamide (Bilivistan), were related to age and sex. Statistical evaluation showed a higher risk for adverse reactions in fertile as opposed to postmenopausal women and in younger men as opposed to older men. This indicated an influence of the steroid levels on the frequency of the side-effects that occurred.

A relation between these findings and serum protein binding of steroid hormones and ioglycamide was proposed. It was decided, therefore, to conduct a biochemical investigation of the interplay between radiographic contrast media and steroid hormones concerning their binding to serum proteins. In Paper II, III and IV, techniques for biochemical investigation of the binding of ioglycamide and steroid hormones to human serum proteins in vitro were described. In these three studies an aqueous two-phase system was used for the separation of unbound ligand from proteins. Ligand molecules were quantified either by UV-light absorption determination (II) or by measuring the irradiation from isotope-labelled ioglycamide (II) and steroid hormones (III ad IV). Competition between the ligands for the binding sites on the proteins under discussion was shown to result in a release of one of the ligands when the level of the other was high.

The role of the binding of these substances to human serum proteins, i.e. albumin and specific steroid binding globulins, was discussed in terms of clinical relevance. In Paper V, gel electrophoresis was performed as a complement to the experiments with the two-phase system in order to detect even the low degree of serum protein binding expected with metrizamide (Amipaque). None, or only little, binding of metrizamide to human serum could be detected. Thus, the primary results concerning binding of metrizamide to proteins was confirmed.

It was emphasized that whole human serum should be used when investigating the protein binding of radiographic contrast media since not only albumin binds them.

On the basis of the results of this investigation, it was concluded that protein binding is involved in the adverse reactions that occur in connection with intravenous injection of radiographic contrast media.
REPORTS CONSTITUTING THE THESIS

This thesis is based on the following papers:

I. WIRELL S.: Adverse reactions at intravenous cholegraphy with ioglycamide. Correlation with the level of circulating sex hormones. Accepted for publication in Acta Radiol. Diagn. (1982).


In the text the papers are referred to by their Roman numerals.
CONTENTS

REPORTS CONSTITUTING THE THESIS .............................................................. 3
INTRODUCTION .................................................................................................... 7
AIMS OF THE STUDY ........................................................................................... 9
METHODOLOGICAL CONSIDERATIONS ............................................................... 9
  Determination of the binding to serum proteins ............................................ 9
  The salivary cortisol concentration - study .................................................. 13
DEFINITIONS ......................................................................................................... 13
  Side-effects, adverse reactions ..................................................................... 13
  Protein binding .............................................................................................. 14
  Age classing ................................................................................................... 15
CLINICAL INVESTIGATIONS .............................................................................. 15
  Previous investigations ................................................................................ 15
  Current clinical study .................................................................................... 16
  Material and methods ................................................................................... 16
  Results ............................................................................................................ 17
EXPERIMENTAL INVESTIGATIONS ................................................................ 19
  Previous investigations ................................................................................ 19
  Techniques ..................................................................................................... 20
  Results ............................................................................................................ 22
GENERAL DISCUSSION ....................................................................................... 27
SUMMARY AND CONCLUSIONS ....................................................................... 33
REFERENCES ...................................................................................................... 34
ACKNOWLEDGEMENTS .................................................................................... 38
PAPER I .............................................................................................................. 41
PAPER II ............................................................................................................ 51
PAPER III .......................................................................................................... 59
PAPER IV .......................................................................................................... 75
PAPER V ............................................................................................................ 87
INTRODUCTION

The use of parenterally administered radiographic contrast media is a prerequisite for several radiodiagnostic examination methods, such as angiography, urography and cholegraphy. During the last decade the introduction of computerized tomography has added new indications for the use of radiographic contrast media.

The use of such compounds is, unfortunately, connected with a risk of unwanted side-effects. It is, however, still obscure what constitutes the toxicity of such substances. Continuous efforts to develop less toxic contrast media have given encouraging results.

Toxic reactions to radiographic contrast media have been ascribed to several phenomena. Complement formation (LASSER 1977), increased peripheral haemodynamic resistance (LINDGREN & SALTZMAN 1962) and effects on the central nervous system by receptors in the hypothalamus (LALLI 1980) have been proposed to be implicated in this connection. In all these circumstances protein binding may be involved.

Parenterally administered radiographic contrast media are partly protein bound during the transport in the blood. There is a direct relation between the toxic effect, the degree of protein binding and the elimination path of each substance (KNOEFFEL & HUANG 1956, LASSER et coll 1962). Thus, the most toxic ones are bound, to a great extent, to serum proteins, whereas less toxic substances are bound to a lesser extent. The protein bound fraction is uncoupled in the liver and excreted with the bile, while the unbound fraction is accessible for filtration in the glomerulae and is excreted with the urine. In both cases side-effects do occur from time to time. The most important immediate side-effects caused by these substances are nausea, vomiting and hypotension (LALLI 1980). These data have been confirmed by the clinical investigation of side-effects to intravenous injection of ioglycamide (I) even if not reported in that paper. The side-effects are ranked here according to their frequency in Table I.
Table I. Frequency of adverse reaction symptoms to intravenous injection of ioglycamide.*

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. of cases displaying the symptom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>45</td>
</tr>
<tr>
<td>Vomiting</td>
<td>28</td>
</tr>
<tr>
<td>Urticaria</td>
<td>19</td>
</tr>
<tr>
<td>Circulation disturbances</td>
<td>10</td>
</tr>
<tr>
<td>Obstructed nostrils or throat</td>
<td>11</td>
</tr>
<tr>
<td>Itching skin</td>
<td>4</td>
</tr>
<tr>
<td>Shivering</td>
<td>2</td>
</tr>
<tr>
<td>Head ache</td>
<td>1</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td>1</td>
</tr>
</tbody>
</table>

Except for urticaria the predominant symptoms are those regarded as being toxic ones.

In animal experiments the relation between steroid and contrast material was studied. Hypotension and vomiting were reported to occur more frequently with cats exposed to iodipamide (Biligrafin forte) if pre-treated with progestin (LINDGREN & SALTZMAN 1962). Cooperation between estrogen and progestagenic substances resulted in a potentiation of the progestagenic effects following exposure to estrogen. An antagonistic effect on the estrogen function exerted by progesterone was also shown by McEWEN et coll (1975). The site of action was supposed to be in the midbrain. It is also generally accepted that radiographic contrast media can penetrate the blood-brain barrier (HOPPE 1959). Further, the area postrema, where chemoreceptors for vomiting producing substances are present, is close to the floor of the fourth ventricle, where there is no blood-brain barrier.

The hypothesis of a release of steroid hormones into a biologically active, non-bound, form by the binding of ioglycamide to serum proteins, was postulated, due to evidence for high steroid hormone level playing a role in the occurrence of adverse reactions. If the hypo-

*Data from Paper I, not previously reported.
thesis could be confirmed, it would help explain the increased risk for adverse reactions in the circumstances under discussion.

That this has not been studied earlier constituted an incentive for this investigation.

AIMS OF THE STUDY

The aims of the present investigation were set

- to study the incidence of adverse reactions to intravenous injection of a cholegraphic contrast compound, ioglycamide (Bilivistan), in relation to age, sex and the different phases of the menstrual cycle,
- to optimize methods for the determination of the binding of radiographic contrast media to serum proteins,
- to study the possible influence of radiographic contrast media on the binding of steroids to human serum proteins and
- to try to relate experimental findings to the adverse reactions recorded in clinical praxis with parenterally administered radiographic contrast media.

METHODOLOGICAL CONSIDERATIONS

Paper I gave evidence for high steroid hormone level being a promoting factor for the occurrence of adverse reactions to intravenous injection of a radiographic contrast compound. That substance was known to bind to serum proteins. It was, therefore, decided to conduct an investigation of the influence of radiographic contrast media on the binding of steroid hormones to serum proteins.

Determination of the binding to serum proteins (Papers II - V)

The two-phase system. The binding of substances e.g. steroids to serum proteins, is usually determined by incubating serum with radioactive
steroids and non-labelled steroids, at different concentrations. After separation of free and bound steroid, the amount in each fraction is determined from the radioactivity. The binding characteristics (binding capacity and association constant) of the binding protein for the substance under discussion can then be obtained from a Scatchard plot (SCATCHARD 1949) using a vectorial subtraction (ROSENTHAL 1967) of the albumin binding.

In this investigation the separation of unbound and bound substance was achieved by an equilibrium partition technique using a two-phase system containing water soluble polymers buffered to a physiological pH (7.4). The two-phase system technique was delineated by ALBERTSSON (1971) and applied for binding studies by SHANBHAG et coll (1973). Minor alterations of the composition of the phase system was proposed by SÖDERGÅRD et coll (1982), where a detailed description of the method was given. A brief review is given here.

When polyethylene glycol and dextran are mixed with water they reject each other and form two separate phases. Due to their specific weights, the dextran phase will form the lower phase and the polyethylene glycol will form the upper phase. The water content of each phase depends on the pH, the temperature and what salts that have been added. In this two-phase system serum proteins and, thereby, the protein-bound substance, the ligand, are partitioned almost totally (more than 99%) into the lower phase; while unbound ligand is partitioned between the phases according to its partition coefficient, which can be determined with the two-phase system in the absence of proteins.

**Fig. 1**

The aqueous two-phase system containing proteins (almost totally distributed to the lower phase) and a ligand which binds, to a certain extent, to the proteins. Equilibrium is established between bound (B) and unbound ligand in the lower phase ($U_{lp}$), and between unbound ligand in the lower phase and unbound ligand in the upper phase ($U_{up}$).
The partition coefficient is the expression for the concentration of ligand in the upper phase divided with the concentration of ligand in the lower phase.

Equilibrium will be established between bound and unbound ligand in the lower phase and between unbound ligand in the upper phase and in the lower phase. It is possible to calculate the amounts of bound (B) and unbound (U) ligand in the lower phase from a sample of the upper phase in which the content of ligand can be determined by measuring the UV-absorption (Paper II) or the radioactivity of isotope-labelled ligand (Papers II, III, IV and V). In Paper II ioglycamide was $^{125}$I- or $^{14}$C-labelled (two different batches). In Papers III and IV progesterone, testosterone and estradiol were $^{3}$H-labelled. In Paper V metrizamide was $^{125}$I-labelled.

The two-phase partition method is rapid and demands only small samples (100μl). It provides optimal possibilities for control of the pH-situation and control over oxidational or other disintegrational problems that could be expected when handling the chemically instable contrast media. The cost of the two-phase system portions is little and the demand for small samples made it possible to perform repeated experiments with numerous observations, thereby increasing the accuracy of the determinations and also providing a possibility for statistical treatment of the results.

Albumin is the multi-potent binding protein in serum. Certain endogenous substances also bind to specific binding globulins. Cortisol and progesterone will thus also bind to transcortin, whereas testosterone and estradiol bind to testosterone-estradiol-binding globulin.

From the calculated content of bound (B) and unbound (U) ligand in the lower phase the ratio B/U versus B is then plotted in accordance with SCATCHARD (1949). Thereby a curve describing the total binding of the ligand can be obtained. The binding to albumin can be obtained from similar experiments with purified albumin or heat-treated serum, where other binding proteins than albumin have been inactivated (PEARLMAN &
By a vectorial subtraction of the albumin binding from the total binding, the specific binding can be obtained (ROSENTHAL 1967). (Fig. 2a). The binding capacity is then calculated from the position of the interception on the abscissa. The association constant is the numerical value of the inclination of the slope. Thus, the binding constants of the specific binding proteins are determined. The apparent association constant for the binding of the ligand to albumin can be calculated from the albumin concentration and the interception point on the ordinate for the albumin binding as described by a line parallel with the x-axis. Altered binding of a ligand can thus be demonstrated if the binding constants change with the presence of another ligand.

Direct measurement of light absorption. (Paper II). An automatic scanning spectrophotometer was used to determine the ultra-violet absorption of solutions of ioglycamide both in water and in serum, from which endogenous hormones had been removed by charcoal absorption in accordance with HEYNS et coll (1967). In both cases steroids were added both to the sample and the reference cuvette and the difference spectrum was recorded automatically. Two continuous scans were carried out in two independent experimental series. Identical curves were obtained and, thereby, even very small alterations of the absorption difference could be considered relevant. Both depression and a shift of the absorption difference maxima towards shorter wave-lengths (blue shift) was noted. Only qualitative information as to whether an altered binding could be shown was obtained, as the calculation on the small alterations would be insignificant.

Gel electrophoresis. (Paper V). Gel electrophoresis was performed in accordance with DAVIS (1964), where a detailed description of the composition of the polyacrylamide gel and the practical steps of the method is given.

It remains unknown how an electrical field influences the binding of radiographic contrast compounds to serum proteins. The risk for errors due to the current would seem to be smaller with non-ionic than with ionic substances, but that such risks can occur should be borne in mind.
The salivary cortisol concentration - study
(Previously not reported.)
Salivary concentration of steroids reflects the free fraction of ste­
roids in serum. Thus, indirect information on the influence of a chole­
graphic contrast compound on the total steroid content and/or the free
fraction of steroid can be obtained by measuring the salivary steroid
concentration.

The first six consecutive patients for intravenous cholegraphy were in­
cluded in the initial part of a current study reported here. As the pa­
tients are their own controls conclusions might be drawn even from this
small material. As a reference group three healthy female volunteers
underwent a similar procedure using saline solution instead of the
currently used cholegraphic contrast compound ioglycamate (Biligram,
Schering AG, Germany). (For details, vide infra).

For methodological considerations in connection with the clinical in­
vestigation (Paper I), vide infra.

DEFINITIONS

Side-effects, adverse reactions
In connection with parenteral administration, in particular intravenous
injection, unexpected side-effects do frequently occur. Among such
effects, toxic and allergic manifestations dominate. In addition, vas­
vagal reactions are seen from time to time and can be immediately re­
cognized by the decrease in the pulse rate. Generally there is no need
for any treatment other than lowering the head of the patient.
Allergic manifestations are urticaria, swelling of the throat or facial
oedema. The pulse rate is generally increased primarily.
Toxic reactions are displayed as nausea and vomiting, increased and
irregular heart rhythm which in severe cases may even develop into ven­
tricular fibrillation and cardiac arrest. These reactions regularly
show fall in blood pressure. Confusion, and even loss of consciousness
may occur as a sign of lowered intracranial perfusion irrespective of the type of reaction that has initiated the blood pressure fall. In the following, vaso-vagal reactions will not be discussed. However, sometimes there are difficulties in separating allergic reactions from toxic ones. Therefore, in the clinical part (Paper I) both sorts of reaction have been discussed as side-effects or adverse reactions.

Protein binding
LANG & LASSER (1967) stated in a report on binding of five different radiographic contrast compounds, that no protein binding, other than that of albumin, could be detected in human serum. This was stated despite the fact, that as judged from the material description in that work, no human serum was used. This spurious statement may be the reason why most binding studies on radiographic contrast media thereafter have dealt with commercial human serum albumin preparations, except for studies made in laboratory animals. The results are then generally described to concern the wider expression: serum protein binding (or lack of such binding).

In this investigation the following terms have been used:

Binding to human blood serum (Paper II) which includes binding to serum proteins as well as such non-specific association to e.g. carbohydrates and serum lipids, that may take place.

Binding to human serum proteins (Papers III, IV and V) where a reservation for the existence of non-specific binding to other substances in human serum than serum proteins has been understood and even proposed (Paper V).

Protein binding: Binding to serum proteins, i.e. albumin, steroid specific binding globulins and other possible binding proteins, mainly globulins, will be referred to under the heading 'protein binding'.

Steroid specific binding globulins: Testosterone-estradiol-binding globulin (TeBG) also known as sex hormone binding globulin (SHBG), transcortin (corticosteroid- or cortisol-binding globulin, CBG). Other specific binding globulins, e.g. thyroxin-binding globulin, have not been taken into account.
Steroid hormones: The steroid sex hormones 5α-dihydrotestosterone, estradiol-17β, 17-hydroxy-progesterone and an adrenocorticosteroid, cortisol, will be grouped under the term 'steroid hormones'.

Age classing
Females
In accordance with SHERMAN et coll (1976) women between the ages of 45 - 56 should be referred to as perimenopausal. In order to exclude, as far as possible, perimenopausal women from fertile or ovulating women, the upper limit for the high level of sex hormones—women has been set as low as at 40 years of age. The rate of ovulations in the age group 50 - 55 years is very low and therefore postmenopausal women have been referred to as those of 51 years of age and older.

Males
According to SCHLEGEL (1974), STEARNS et coll (1974) and READ et coll (1981) a continuous decrease in the testosterone level takes place after the age of 30. No obvious drop is present in this curve. Even if huge personal variations occur, the level is generally very low from the age of 70. In order to make an age grouping that includes younger men (high testosterone level) the borderline, separating those from elderly men, has been set at the age of 70.

CLINICAL INVESTIGATION (Paper I, and a current study)

Previous investigations
In the early sixties, a bile-excreted, radiographic contrast compound, iodipamide (Biligrafin, Cholografin), was introduced for use intravenously. It is still in use today despite it being one of the most toxic contrast media.

In the mid-sixties, a new cholangiographic contrast compound, ioglycamide (Bilivistan), was introduced. It was considered to be less toxic than iodipamide (BRISMAR et coll 1971). The connection between side-effects after intravenous injection of iodipamide and sex hormone
levels was reported by LINDGREN & SALTZMAN (1962). The protein binding of contrast media and the interaction with other protein bound substances, e.g. bilirubin and salicylates, were investigated and reported in a widely spread paper by LASSER et coll (1962). Further studies on the influence of steroid sex hormones on side-effects to ioglycamide were done by LINDGREN et coll (1974). At that time, the first report of lethal reaction following intravenous injection of ioglycamide in a patient suffering from macroglobulinæmia (BAUER et coll 1974) turned up. Protein binding of contrast compounds had, before that, been considered to be synonymous with albumin binding (LANG & LASSER 1967), but now globulins had to be taken into consideration. Potentiation of pentobarbital anesthesia by competitive protein binding of, amongst other contrast media, Cholografin (iodipamide), was reported by LASSER et coll in 1963. An increased toxic propensity displayed by iodipamide at pentobarbital anaesthesia was also reported in that work.

Steroid hormones had long been known to bind to several protein fractions, but the connection between protein binding of sex hormones and protein binding of radiographic contrast media had still to be investigated.

Current clinical study*
Measurements of salivary steroid concentrations correlate well with the serum levels of non-protein-bound hormone (SMITH et coll 1979, BAXENDALE et coll 1980). This fact made it possible to determine the level of free steroid before and after the administration of ioglycamate (Biligram, Schering AG, Germany), which is the cholegraphic contrast compound currently used in this X-ray Department. The results of the first six cases where the salivary cortisol concentration was measured in a current clinical study will be described.

Materials and methods (Paper I)
The material consisted of 900 consecutive patients for intravenous cholegraphy. Out of them, 39 were excluded from the material. Some simply refused to undergo the examination, in others examination was not performed due to manifest asthmatic seizure when they turned up. A

*Not previously reported.
third group was excluded due to incomplete information in the form necessary for the study. Of the remaining 861 patients, the first 500 were included in a previous report on side-effects seen in connection with intravenous cholecography (BRISMAR et coll 1971). They were all examined with an intravenous injection of 20 ml of ioglycamide (Bilivistan, 0.5 g/ml, Schering AG, Germany) at a rate of 4 ml/min. Abberation in the injection rate was noted. Amongst other parameters the first day of the preceding menstruation was noted in fertile women. Adverse reactions that occurred were described in general terms regarding symptoms and the presumed diagnosis for the type of reactions was stated. The time of onset of the reactions was related to the starting-time of the injection.

For age grouping, see DEFINITIONS.

Among fertile women, not taking oral contraceptives, the ranking of the material was done in order to determine classes with defined sex hormone levels. This was done on the basis of the onset of the preceding menstruation. The examination day was related to that day, in each case.

(The current clinical study.) In six cases the salivary concentration of cortisol was measured immediately before and after the infusion of 100 ml ioglycamate 0.17 g/ml (Biligram, Schering AG, Germany). The patients were instructed to spit directly into vials until a volume of 1 - 2 ml of saliva was obtained. The time elapsing between the collection of the two samples of saliva was 35 - 45 minutes. The samples were centrifuged and stored at -20°C until analysed in accordance with the routine for serum cortisol analysis (radioimmunoassay, RIA) at the Department of Clinical Chemistry at the University Hospital in Umeå. The control was performed on three healthy female volunteers using 100 ml of saline instead of the ioglycamate solution.

Results (Paper I)

Of the 861 cases 107 (12.4%) displayed adverse reactions in connection with the cholecography. No difference between sexes could be demonstrated.
The reactions were generally seen during the injection of the contrast compound. In one case the first symptom of reaction (complaint of obstruction in the throat) was noted 30 minutes after the injection had been started. In another case urticaria developed about 13 minutes after the injection had been commenced.

Age classification (see DEFINITIONS) showed a considerably higher frequency of adverse reactions in younger males (16.2%) than in elderly ones (1.7%). In women the difference was less striking, but about half as many postmenopausal women (17.0%) than fertile women (9.0%) reacted adversely.

The fertile women were divided into three groups, related to the menstrual cycle. Those in the first two thirds of the cycle reacted with a frequency similar to that of postmenopausal women. The highest frequency of reaction seen in any of the groups was that of the third part of the menstrual cycle (21.2%) (Table II).

Table II. Frequency of adverse reactions related to parts of the menstrual cycle and their progesterone levels.

<table>
<thead>
<tr>
<th>Day after onset of menstruation</th>
<th>0 - 9</th>
<th>10 - 19</th>
<th>20 - 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>60</td>
<td>48</td>
<td>33</td>
</tr>
<tr>
<td>Side-effects</td>
<td>6 (10.0%)</td>
<td>5 (10.4%)</td>
<td>7 (21.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Progesterone levels* ng/ml</th>
<th>0.3 - 0.8</th>
<th>0.3 - 2.0</th>
<th>2.0 - 20</th>
</tr>
</thead>
</table>

*in accordance with ABRAHAM et coll (1972).

A detailed analysis of the results is given in Paper I.
(The current clinical study.) The salivary study showed that the cortisol concentration increased 3 times or more in five of the six cases. In one case only slight increase was noted (Table III). No change was observed in the three controls.

Table III. Salivary cortisol concentration alteration in connection with infusion of ioglycamate (Biligram). Controls beneath (cases 7 - 9).

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Salivary concentration of cortisol</th>
<th>Increase of cortisol concentration (factor)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before infusion (nM)</td>
<td>After infusion (nM)</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>5*</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>4*</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>5*</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>8*</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>9*</td>
<td>31</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8*</td>
<td>4*</td>
</tr>
<tr>
<td>8</td>
<td>4*</td>
<td>6*</td>
</tr>
<tr>
<td>9</td>
<td>12</td>
<td>10*</td>
</tr>
</tbody>
</table>

*Uncertain figures at that low level.

EXPERIMENTAL INVESTIGATIONS
(Ioglycamide in Papers II, III and IV; metrizamide in Paper V)

Previous investigations
It has long been known that competition takes place between various molecules with respect to the binding sites of proteins. A review on
this matter was made by KLOTZ (1953). Organic ions, such as salicylates and sulfonamides were known to dissociate bilirubin from albumin by competitive binding (ODELL 1959). Protein binding of radiographic contrast media and the implication of that phenomenon was discussed in terms of clinical relevance by LASSER et coll (1962). That work hinted at a competition between radiographic contrast media and bilirubin for the binding to the albumin molecule.

Competitive protein binding of an anesthetic compound, pentobarbital, resulted in a potentiation of the narcotic effect of that substance in the presence of protein bound contrast media in rats (LASSER et coll 1963). In that paper increased toxicity of Cholografin under pentobarbital anesthesia was also noted.

In the work by LINDGREN & SALTZMAN (1962) cats were found to exhibit fall in blood pressure and vomiting if pretreated with progestin before administration of iodipamide (Biligrafin forte). Similar experiments were performed using ioglycamide instead of the more toxic iodipamide (LINDGREN et coll 1974). The hypothesis of an interaction between protein bound contrast media and sex hormones was proposed. Protein binding of bile-excreted radiographic contrast media was supposed to be the reason for toxic reactions towards such substances.

Metrizamide (Amipaque, Nyegaard & Co., Norway) has been carefully presented in Supplements No. 335 (1973) and No. 355 (1977) to Acta Radiologica. It was shown that metrizamide does not bind to serum proteins in rodents or cats (SALVESEN 1973, GOLMAN 1973). Protein binding studies performed with commercial human serum albumin (SALVESEN & FREY 1973) failed to reveal any binding of metrizamide. Other serum proteins were not included in these experiments and binding to globulins remained to be investigated.

Techniques
The experiments performed in this investigation were mainly done as separation of ligands in an aqueous two-phase system (Papers II - V).
Complementary experiments were performed as a direct measure of the UV-light absorption (Paper II) and as separation of protein fractions with gel electrophoresis (Paper V). Detection of unbound ioglycamide, metrizamide or steroid hormones was performed by liquid scintillation or direct measurement of the irradiation, depending on the energy of irradiation emitted by the isotopes labelling the substances.

The two-phase system technique has been outlined by ALBERTSSON (1971). Its application for binding studies involving plasma proteins was worked out by SHANBHAG et coll (1973). In that work a detailed description of the method is presented. Minor alterations of the composition of the phase system was proposed by SÖDERGÅRD et coll (1982).

In the Papers II - V brief descriptions of that method were given. A short recapitulation could be of value, though.

In a mixture of aqueous solutions of dextran and polyethylene glycol in certain proportions, two phases are formed due to the mutual exclusion of the polymers. The dextran will concentrate in the lower phase and the polyethylene glycol in the upper phase. In such two-phase systems partition of proteins depends on molecular weight, total concentration of the two polymers, the type and concentration of the salt included in the system, as well as pH and temperature.

In the system used in this investigation, the serum proteins are distributed almost totally to the lower phase. The steroid hormones, on the other hand, have a partition coefficient (concentration in the upper phase/concentration in the lower phase) between 1.5 to 3.0. When a protein and a ligand that binds to the protein are partitioned together, a fraction of the ligand, depending on its association constant, will be bound to the protein. The protein bound part of the ligand will be found, together with the protein, in the lower phase. The unbound part of the ligand is distributed between the phases in accordance with its partition coefficient. From a determination of the unbound ligand concentration in the upper phase, it is possible to calculate the amount of the protein bound ligand in the lower phase. A principle scheme is given in Fig. 1.
The gel electrophoresis experiments (Paper V) were performed in accordance with DAVIS (1964), where the exact composition of the polyacrylamide gel as well as practical instructions are given.

Results (Paper II)
The need for careful attention to oxidation, and other forms of disintegration, was necessary as deformation of the UV-light absorption curve occurred during the automatic recording of the UV-spectrum. This problem could be overcome by performing the experiments with ioglycamide at a low temperature (+2°C), under nitrogen and protected from daylight.

A blue shift and lowered absorption of UV-light was observed in the presence of steroid sex hormones, particularly progesterone at a concentration comparable with that in the luteal phase of the menstrual cycle. The presence of cortisol did not result in any blue shift, but in a depression of the absorption only.

When ioglycamide was introduced to serum after the addition of progesterone, the association constant (binding constant) increased a little, but if the substances were added in the opposite order a slight decrease occurred. This was supposed to be due to an altered conformation of the protein molecules. In the following experiments the contrast compounds were always introduced after the addition of the steroid hormones (Papers III - V).

In Paper III the effect of ioglycamide on the binding of progesterone to human serum proteins was investigated. It was shown that progesterone was released from serum proteins, at least in a high ioglycamide concentration, which was similar to that present in a clinical examination situation, where 20 ml of the contrast are mixed with 5 litres of blood. The binding curves for progesterone could be described with an exponential function. A virtual competition between ioglycamide and progesterone concerning their respective binding to serum proteins was shown (Papers II, III), (Table IVa, Fig. 2a - b).
Fig. 2a. Scatchard plot and vectorial subtraction (Rosenthal) of the binding of progesterone. 
T = the curve for the total binding of progesterone. 
A = the line for the albumin binding of progesterone. 
S = the line for the specific binding (transcortin binding) of progesterone. 
These curves and lines are interrupted in Fig. 2b, where they serve as references.

Fig. 2b. Scatchard plots of the total binding and the albumin binding as well as the vectorially subtracted specific binding of progesterone. The continuous curve and lines represent the decreased binding of progesterone in the presence of a high concentration of ioglycamide. That concentration is still relevant in an in vivo clinical examination situation.
In Paper IV the effect of ioglycamide on the binding of testosterone and estradiol was investigated. In the testosterone-case the serum was pretreated with cortisol. This was done in order to demonstrate the effect of ioglycamide on testosterone-estradiol-binding globulin only. Testosterone is also bound to transcortin, but the effect of ioglycamide on that specific binding globulin was specially demonstrated in the progesterone-case (Paper III). The graphic results of the experiments with first none and then three different concentrations of ioglycamide are given in Figures 3a and b. In Tables IVa-c the data for binding to albumin and to specific binding globulins respectively are presented as association constant and binding capacity for the different steroid hormones (Papers III and IV). The observed differences both in the figures and in the parameters recorded in the tables are due to the influence of ioglycamide. The testosterone-estradiol-binding globulin parameters are less influenced by ioglycamide than those of transcortin.

![Fig. 3a. Testosterone](image)
![Fig. 3b. Estradiol](image)

Scatchard plot of the ratio B/U versus B gave the curves for the total binding of the respective steroid to serum proteins. The horizontal lines represent the albumin binding of each steroid. The oblique lines represent the binding of steroid to TeBG.

No ioglycamide present: 

0.05 mg ioglycamide/2.5 g phase system: 

0.5 mg ioglycamide/2.5 g phase system: 

5.0 mg ioglycamide/2.5 g phase system:
Table IVa. Binding characteristics of progesterone for the binding to albumin and transcortin at different concentrations of ioglycamide.

<table>
<thead>
<tr>
<th>Ioglycamide</th>
<th>Albumin</th>
<th>Transcortin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apparent association constant</td>
<td>Association constant</td>
</tr>
<tr>
<td></td>
<td>$x 10^{-4}$</td>
<td>$x 10^{-7}$</td>
</tr>
<tr>
<td></td>
<td>(M)</td>
<td>(M)</td>
</tr>
<tr>
<td>0</td>
<td>6.24</td>
<td>8.17</td>
</tr>
<tr>
<td>0.05</td>
<td>6.13</td>
<td>8.09</td>
</tr>
<tr>
<td>0.5</td>
<td>6.08</td>
<td>7.52*</td>
</tr>
<tr>
<td>5.0</td>
<td>5.67*</td>
<td>7.00**</td>
</tr>
</tbody>
</table>

* Significantly ($p < 0.05$) different from the non-ioglycamide situation
** Significantly ($p < 0.01$) different from the non-ioglycamide situation

Table IVb. Binding characteristics of testosterone for the binding to albumin and TeBG at different concentrations of ioglycamide.

<table>
<thead>
<tr>
<th>Ioglycamide</th>
<th>Albumin</th>
<th>TeBG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apparent association constant</td>
<td>Association constant</td>
</tr>
<tr>
<td></td>
<td>$x 10^{-4}$</td>
<td>$x 10^{-9}$</td>
</tr>
<tr>
<td></td>
<td>($M^{-1}$)</td>
<td>($M^{-1}$)</td>
</tr>
<tr>
<td>0</td>
<td>6.72</td>
<td>1.87</td>
</tr>
<tr>
<td>0.05</td>
<td>6.76</td>
<td>1.66</td>
</tr>
<tr>
<td>0.5</td>
<td>6.36</td>
<td>2.12</td>
</tr>
<tr>
<td>5.0</td>
<td>5.24</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Table IVc. Binding characteristics of estradiol for the binding to albumin and TeBG at different concentrations of ioglycamide.

<table>
<thead>
<tr>
<th>Ioglycamide</th>
<th>Albumin</th>
<th>TeBG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apparent association constant</td>
<td>Association constant</td>
</tr>
<tr>
<td></td>
<td>$x 10^{-4}$</td>
<td>$x 10^{-9}$</td>
</tr>
<tr>
<td></td>
<td>($M^{-1}$)</td>
<td>($M^{-1}$)</td>
</tr>
<tr>
<td>0</td>
<td>8.00</td>
<td>1.11</td>
</tr>
<tr>
<td>0.05</td>
<td>7.88</td>
<td>1.05</td>
</tr>
<tr>
<td>0.5</td>
<td>7.74</td>
<td>1.14</td>
</tr>
<tr>
<td>5.0</td>
<td>7.88</td>
<td>1.24</td>
</tr>
</tbody>
</table>
In Paper V the two-phase system experiments failed to reveal any protein binding of metrizamide. The gel electrophoresis, on the other hand, showed a slightly increased activity, representing metrizamide in connection with serum proteins (Fig. 4). The relevance of this finding is obscure (vide infra).

![Fig. 4. Gel electrophoresis of human serum in the presence of $^{125}$I-labelled metrizamide. Radioactivity in the slices (1.6 mm) cut from the gel-column (total length: 58.4 mm), expressed as counts per minute - CPM -. The x-axis corresponds to the gel-column on the photograph below the diagram. The stained sections of the gel-column show the different fractions of human serum proteins. The horizontal line in the histogram indicates the background radiation of 13 CPM.](image)

The results pertaining to the altered binding of ioglycamide in the presence of steroid hormones are analysed in Paper II. A detailed analysis of the results of the influence of ioglycamide on the binding of progesterone is presented in Paper III and that of testosterone and estradiol in Paper IV.

The results of the investigation of the metrizamide binding are given in detail in Paper V.
GENERAL DISCUSSION
(Papers I - V and the unpublished salivary cortisol study)

From previous reports on clinical (BRISMAR et coll 1971) and experimental (LINDGREN et coll 1974) investigations of side-effects occurring in connection with intravenous injection of a cholegraphic contrast medium, ioglycamide (Bilivistan), it is known that the frequency of such reactions is of the order of 10 per cent, but can be elevated by the influence of other protein-bound substances, (e.g. salicylates and bilirubin) (LASSER et coll 1962, LINDGREN & SALTZMAN 1962, LASSER et coll 1963, LINDGREN et coll 1974). The connection between iodipamide and progesterone (LINDGREN & SALTZMAN 1962) or ioglycamide and progesterone (LINDGREN et coll 1974), as mutual promotors of such effects, was the direct reason for starting this investigation.

Extension of the material of BRISMAR et coll (1971) to include almost 900 patients undergoing cholegraphy permitted statistical evaluation of the adverse effects following the injection of ioglycamide.

The results of this investigation showed an elevated risk for adverse reaction to occur in younger males and in fertile women during the luteal phase of the menstrual cycle. Thus, elevated risks for adverse reactions were present in situations with high levels of testosterone and progesterone (Paper I). These substances bind both to albumin and to cortisol-binding globulin (transcortin). Testosterone also binds to testosterone-estradiol-binding globulin.

It was also known that ioglycamide binds to serum proteins. The expressions: serum proteins and albumin have been used synonymously since LANG & LASSER (1967) stated that no binding for five different contrast compounds to serum proteins except to albumin, could be proved. This spurious statement was presented despite the fact that no other human serum protein than albumin was examined in that work. Hence, subsequent investigations of ioglycamide and other contrast compounds have almost exclusively dealt with purified human serum albumin in in vitro experiments or toxic animal reactions.
The connection between sex hormones and adverse reactions at intravenous cholegraphy was undoubtedly difficult to explain if the high capacity binding property of albumin only was involved. The aim of the first experimental work (Paper II) was therefore primarily set to investigate the influence of a number of different hormones on the binding of ioglycamide to serum proteins in order to detect, whether ioglycamide could be released from proteins other than albumin. The applicability of the two-phase partition method in this field of investigation, as well as the management of disintegrational problems that could be expected to arise with the chemically instable ioglycamide, would be achieved, as side-effects.

The release of ioglycamide was obvious but too little to be determined quantitatively. This seems logical when considering the vast disproportions between the concentration of steroid hormones and that of ioglycamide in an in vivo situation (Tables V, VI).

Table V. Concentration of albumin, ioglycamide and progesterone in human blood.

<table>
<thead>
<tr>
<th>Albumin (M)</th>
<th>Ioglycamide (M)</th>
<th>Progesterone (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(7.7 \times 10^{-3})</td>
<td>(1.4 \times 10^{-2})</td>
<td>(9.5 \times 10^{-9} - 6.4 \times 10^{-8})</td>
</tr>
</tbody>
</table>

General order of approximated figures

\(10^{-2}\) \(10^{-2}\) \(10^{-8} - 10^{-7}\)

Table VI. Content of albumin, ioglycamide and progesterone in the total (5 litres) blood volume.

<table>
<thead>
<tr>
<th>Albumin (g)</th>
<th>Ioglycamide (g)</th>
<th>Progesterone (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2.25 \times 10^{2})</td>
<td>(1.0 \times 10^{1})</td>
<td>(1.5 \times 10^{-6} - 1.0 \times 10^{-4})</td>
</tr>
</tbody>
</table>
In Paper III release of progesterone from transcortin and albumin by ioglycamide was demonstrated.

As the concentration of ioglycamide in vivo is equimolar with that of albumin and as there are multiple binding sites on the albumin molecule for progesterone, just the occupation of the binding sites by ioglycamide could not alone be responsible for the decreased binding constants for progesterone. It is, however, possible that an allosteric change (altered form of the molecule) in albumin, following the binding of ioglycamide could affect the degree of progesterone binding. A disturbed binding of progesterone by influence of ioglycamide on the water 'structure' would constitute another possible explanation for the decreased progesterone binding.

Both these arguments could be applied to the progesterone-transcortin binding as well as to the release of testosterone and progesterone from albumin (Paper IV).

An even more pronounced effect of the latter mechanism would be expected in vivo compared with the effect in the two-phase system as the serum is diluted about 10 times with water in such a system.

Such a release in vivo would cause an elevation of the total progesterone content to extra-physiological values. Such an elevation could, undoubtedly, be responsible for the adverse reactions seen in connection with the injection of 20 ml of ioglycamide at intravenous cholegraphy. In Paper IV release of testosterone and estradiol by ioglycamide was demonstrated. The albumin-binding of testosterone decreased as did that of progesterone, whereas the albumin-binding of estradiol did not change. An alteration of the albumin molecule in the presence of ioglycamide, as proposed in Papers II and III, does not seem to affect the binding sites on the albumin-molecule for estradiol significantly.

The transcortin-binding of testosterone was blocked by the addition of cortisol in the testosterone-experiments. The decrease of the binding of testosterone and estradiol to testosterone-estradiol-binding globulin was little.
On the basis of the results from Papers II - IV it was decided to investigate the clinical relevance of the information on the influence of ioglycamide (Bilivistan) on the binding of steroid hormones obtained by the in vitro-experiments. The side-effects seen in connection with cholegraphy (Paper I) resemble those occurring at the onset of pregnancy. To the best of the writer's knowledge, such reactions have not been reported to be related to increased testosterone levels. The experimental and clinical results reported by LINDGREN et coll (1974) indicate the role of progesterone in this connection.

Both progesterone and cortisol are bound to albumin and transcortin in serum (WESTPHAL 1971). The concentration of cortisol is of the order of 100 times that of progesterone in serum. It would, therefore, seem advantageous to study the influence of ioglycamide on the binding of steroids to albumin and transcortin by using cortisol instead of progesterone. The salivary cortisol concentration was found to increase considerably following the infusion of ioglycamate (Biligram, Schering AG, Germany), which is actually the same substance as ioglycamide. If a choice is to be made between the contrast media (ioglycamide, Bilivistan and ioglycamate, Biligram), it must be taken into consideration that although Biligram is ready for infusion it is considerably more expensive than Bilivistan.

The increased steroid level in saliva reflects directly the level of free steroid in serum (SMITH et coll 1979, BAXENDALE et coll 1980). The finding of increased cortisol concentration in saliva confirms the idea that ioglycamide causes a release of steroid hormones from serum proteins as indicated by the ioglycamide induced reduction of the binding constants shown in the in vitro experiments.

This investigation has studied the serum protein binding of contrast media and steroid hormones and the interplay between the respective protein binding of these substances. Other implications of protein binding, such as contrast media binding to the 'acute phase' reactive proteins of the complement and coagulation systems, has not been taken
into consideration in this study. Complement activation studies are currently performed in other laboratories (INVEST. RADIOL. 1980) and interesting results have recently been reported e.g. in Supplementum 15 to Investigative Radiology (1980). In a recently started study the preliminary results confirm the idea that ioglycamide can cause a release of steroid hormones. The in vivo dignity of such a release reaches an alarming magnitude and must, therefore, be considered in a discussion on the background for adverse reactions to occur.

In Paper V, gel electrophoresis and separation with the aqueous two-phase system were used to complete the primary works with equilibrium dialysis by SALVESEN & FREY (1973).

The need for the use of whole human serum when investigating the protein binding of a radiographic contrast medium constituted the major reason for this study. Now that three methods have failed to show any significant protein binding of metrizamide, the opinion that no such binding takes place, is better founded.

In a recent work by MÜTZEL et coll (1980), a non-neglectable binding of metrizamide was reported. Those experiments, performed as equilibrium dialysis, were made at room temperature for 4 hours and at pH 7.0. Even if performed with human plasma, the experimental situation in that work stands in opposition to the demands for physiological pH and the avoidance of disintegrational factors experienced in this laboratory. In the light of this, and the probable non-specific association experienced with gel electrophoresis (V), there are reasons to believe that the results by MÜTZEL et coll (1980) were erroneous in the respect of protein binding.

During the last few years new radiographic contrast compounds for cholegraphy (iotroxamide, Biliscopin, Schering AG, Germany) and for general purposes, e.g. angiography, urography and for use in the subarachnoid space (iohexol, Nyegaard & Co., Norway), have been presented (DORAN & BELL 1980, Supplement No. 362 to Acta Radiologica 1980). No convincing reports of reduced toxicity of iotroxamide in comparison
with other cholegraphic contrast media have been presented. Iohexol, on the other hand, has been shown even less toxic, in animal experiments, than metrizamide, the preceding non-ionic contrast compound from the same laboratories. Metrizamide has a poor chemical stability and is expensive to produce. Iohexol does not have these disadvantages. Only little protein binding affinity has been reported with those last two substances.

If it were possible to alter the molecule of iohexol to a more protein bound substance, one would most likely obtain a bile-excreted, low toxic substance.

Further studies of the reason why ioglycamide, or related substances, are toxic, might lead to the knowledge of how a cholegraphic substance of less toxic propensity should be composed. It still remains obscure how ioglycamide influences e.g. the steroid receptors, the membrane function and if it influences other hormone systems e.g. the thyroxine/thyroxine-binding globulin relation. It is planned, therefore, to continue the binding studies, commenced here, to investigate these obscurities.

The influence of radiographic contrast media on the level of active steroid hormones has now been demonstrated. It should be possible to develop a laboratory method to predict the occurrence of adverse reactions that are related to this phenomenon by a laboratory test. It should be possible to determine which would be the contrast substance of choice in each case.

It would also be desirable to be able to prevent or to treat complications caused by the administration of the contrast media with a substance which inactivates or weakens the effect of the unbound sex hormones.
SUMMARY AND CONCLUSIONS

Adverse reactions occurring following intravenous injection of ioglycamide were related to both age and sex in a clinical study of 861 consecutive cholegraphy examinations. Increased frequency of such reactions were noted in young men and fertile women, especially during the luteal phase of the menstrual cycle. Elevated progesterone and testosterone levels were considered to be an underlying reason for the high frequency of such reactions.

Experimental studies of the altered binding of the sex hormones to transcortin and testosterone-estradiol-binding globulin revealed release of, particularly, progesterone from human serum proteins including albumin and specific steroid binding globulins. The clinical relevance of the increased free fraction of progesterone was discussed.

For comparative reasons the binding of a urine-excreted radiographic contrast compound, metrizamide, was studied. The lack of protein binding of that substance, within the sensitivity borders of approximately one per cent with the two-phase system, was confirmed.

The finding of increased risks for the occurrence of adverse reactions in patients with high sex hormone levels were discussed in terms of clinical implications. The role of the protein binding in connection with such reactions remains unclear. Protein binding may still be considered a link in a complex system of partial phenomena that, together, constitute the pathway for adverse reactions to occur.

Thus it would be concluded that

1. The frequency of adverse reactions in connection with intravenous cholegraphy with ioglycamide is increased when the sex hormone levels are elevated.

2. There is a prerequisite of careful attention to oxidation and other disintegrational factors as well as pH when investigating the protein binding of contrast media. The use of whole human serum as a
protein pool is of utmost importance as such compounds bind not only to albumin but to globulins also. The difference between species concerning their different tolerance for, and affinity to bind, contrast compounds constitute additional reasons for careful restraint in the extrapolation of results obtained with animal experiments to the human situation.

3. The apparent association constant for the albumin binding of progesterone and testosterone can be decreased by the presence of ioglycamide at high concentration. The binding capacity for the transcortin binding of progesterone and that of testosterone-estradiol-binding globulin binding of testosterone and estradiol can be decreased by the presence of ioglycamide. The non-ionic contrast compound metrizamide does not bind significantly to serum proteins.

There are no contradictory evidences for the correctness of these results.

4. The altered binding constants can be the expression for such altered binding of steroid sex hormones that is responsible for increased risk for the adverse reactions under discussion to occur when the sex hormone levels are high. It seems to be obvious that the protein binding of contrast media is to a non-neglectable extent responsible for the toxicity of the radiographic contrast compounds. The background for the toxicity seems, however, to be complex: the protein binding being one of several different factors.

REFERENCES


McEWEN B.S., PLAPINGER L., CHAPTAL C., GERLACH J. and WALLACH G.: Role of fetoneonatal estrogen binding proteins in the association of estrogen with neonatal brain cell nuclear receptors. Brain Research 96 (1975), 400.


ACKNOWLEDGEMENTS

I wish first to express my gratitude to the head of my Department, Professor Georg-Fredrik Saltzman, who initiated this investigation. Without his encouragement, support and knowledge in this field of radiology, this work would never have been completed, - or even started.

My friend and co-author Ragnar Södergård, Ph.D., has introduced me into endocrinological biochemistry and scientific theory. He has taught me the basics of laboratory work and helped me with practical problems, as they occurred to me, even if not to him. My thanks are due to him for support in 4/5 of this work, the part of it that has not been performed in the mornings.

Thanks are due to Gunnar Selstam, Ph.D., head of the Section for Endocrinology, Department of Physiology in Umeå, as co-author and general adviser. Hans Carstensen, former Assistant Professor of that Department, Vasant Shanbhag, Ph.D. and Professor Per-Åke Albertsson, former head of the Department of Biochemistry in Umeå, who convinced me of the advantages of the method for studying these problems and which they had elaborated. Vasant Shanbhag also revised the manuscript of this thesis.

I wish to express my gratitude to Lennart Gustavsson, M.A., and Erik Arvidsson, B.A., for the statistical planning and evaluation, and all my colleagues at the Department of Radiology in Umeå for accepting more than their normal share of the routine work during periods of this investigation.

In particular I want to thank Professor Bengt Liliequist who arranged my routine work at the Department of Neuroradiology to suit the requirements of the investigational work. I also wish to mention the personell at the Departments of Physiology and Radiology for their contribution in this connection, as well as Ulf Rosén, Ph.D., at the Department of Clinical Chemistry in Umeå for assistance in the saliva cortisol tests.
Miss Christina Eriksson, Miss Lolomai Örnehult, and Miss Teija Liikkanen gave skilful secreterial assistance, the latter with utmost high speed and good spirit during the last, stressing period of this work.

Mrs Grace Lundberg translated Paper II into English. Dr. and Mrs Sherdil Nath, M.D., my friends, revised the English manuscripts (Papers I, III, IV and the thesis). My very good friend Stefan Emdin, M.D., Ph.D., gave valuable advise (Papers I, III, IV and V) and revised the English manuscript (Paper V).

The Directory Board of Umeå Health Care District have through their Personal Section offered me and my family habitat in direct neighbourhood with the Hospital, thereby facilitating late evenings work. This opportunity cannot be overestimated.

Others have contributed. They know I have not forgotten them.

Some have suffered from my work with this thesis. To my children Nils Johan (6), slalom-skier, fiddler, hockey-player and LEGO-engineer, Kajsa (5), ballet-dancer, slalom-skier and painter, and to Signe (1.5), a pearl with humoristic sense, I wish to say: go on being the wonderful human creatures that you are.

To my wife Marianne: Thank you!

This investigation was supported by grants from the Medical Faculty, University of Umeå and the Swedish Medical Research Council (grant No. B-81-04X-05653).