Bedingungen der Bestimmungsmethode eine Farbintensität, die ungefähr 25% einer gleichkonzentrierten Indicanlösung ausmacht, was als Deutung der grünen Farbe bei Untersuchungen des Urins einiger Leberkranker angeführt werden kann.

Haltbarkeit der Farbe

Nach Lösen in Methanol nimmt die Farbintensität innerhalb 30 Minuten um rund 5% ab. Es soll deshalb sofort nach dem Lösen abgelesen werden.

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Separation of Cystine-Amino-Peptidase and Leucine-Amino-Peptidase and their determination in pregnant and nonpregnant women

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Serum leucine-aminopeptidase (LAP) and cystine-aminopeptidase (CAP) determinations have been made using a chemical method based on enzymatic hydrolysis of l-leucine-β-naphthylamide-hydrochloride and cystine-di-β-naphthylamide, respectively. Fractionation of serum proteins by gel filtration on "Sephadex-G 200" has been used for separation of LAP and CAP (oxytocinase). — CAP which appears during pregnancy only and LAP present in all sera, hydrolyse both substrates, LNA and CDNA. LAP has been found to cleave LNA 74 times faster than CDNA and CAP has been confirmed to hydrolyse LNA 11 times faster than CDNA.
CAP-activity found in non-pregnant serum with small, but significant values, is due to LAP hydrolysing CDNA. Comparing these CAP-activity values in normal individuals with CAP-activity levels at term, only a 13-fold increase of CAP-activity can be recovered. Subtraction of CAP-activity due to LAP from total CAP-activity in early pregnancy reveals against net CAP values at term an increase of CAP activity which is comparable to ratios found with oxytocinase determinations based on a bioassay methods. — Separation of CAP from LAP on "Sephadex" gel dilutes small enzyme concentrations as appear during the first half of gestation to such an extent as to make pooling and concentration procedures necessary in order to achieve appreciable accuracy for CAP assays.
The slow increase of CAP during the first half of gestation, the variation of LAP-activity in normal individuals and the mutual substrate unspecificity of both enzyme render LAP and CAP assays invalid for the diagnosis of early pregnancy.

Bestimmungen von Serum-Leuzin-Aminopeptidase (LAP) und Cystin-Aminopeptidase (CAP) wurden mit Hilfe eines chemischen Verfahrens vorgenommen, das auf der enzymatischen Hydrolyse von l-Leuzin-β-naphthylamid-hydrochlorid und entsprechend Cystin-di-β-naphthylamid beruht. Zur Trennung von LAP und CAP (Oxytocinase) wurde die Fraktionierung der Serumproteine mit Hilfe von Gelfiltration an „Sephadex-G 200" verwendet. CAP, welches nur während der Gravidität erscheint, und LAP, das in allen Seren vorhanden ist, hydrolysieren sowohl LNA wie CDNA. Es wurde gefunden, daß LAP LNA 74mal schneller aufspaltet als CDNA und CAP hydrolysiert LNA 11mal schneller als CDNA.
CAP-Aktivität, welche mit geringen aber eindeutigen Werten im Serum von Nichtgraviden nachgewiesen wird, beruht auf LAP, welche CDNA hydrolysiert. Vergleicht man diese CAP-Aktivitätswerte bei normalen Individuen mit CAP-Aktivitätswerten am Termin, so kann nur ein 13faches Ansteigen der CAP-Aktivität festgestellt werden.
Zieht man die durch LAP verursachte CAP-Aktivität vom Gesamt-CAP-Aktivitätswert bei Schwangerschaftsbeginn ab, so zeigt sich gegen reine CAP-Werte am Termin eine Zunahme der CAP-Aktivtät, welche vergleichbar ist den Werten, die mit Oxytocinase-Bestimmungen gefunden wurden, die auf biologischer Methodik beruhen. — Trennung der CAP von LAP an „Sephadex-gel" verdünnt kleine Enzymkonzentrationen, wie sie während der ersten Hälfte der Schwagerschaft erscheinen, in einem solchen Ausmaß, daß Sammeln und Konzentrierungsmaßnahmen notwendig sind, um wirkliche Zuverlässigkeit für CAP-Bestimmungen zu erhalten. Die langsame Zunahme von CAP während der ersten Hälfte der Schwangerschaft, die Streuung der LAP-Aktivität bei gesunden Individuen und die wechselseitige Substrat-Unspezifität beider Enzyme machen LAP- und CAP-Bestimmungen für die Frühdiagnose der Schwangerschaft ungeeignet.

The inactivation of oxytocin by human and rhesus monkey plasma during pregnancy has been attributed to an enzyme called *oxytocinase* (1). Studies on oxytocinase have been extended by several groups of investigators (2—5). In the above studies the amount of oxytocin not inactivated was determined subsequently by bioassays.

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In 1957, TUPPY and NESVADBA (6) reported that oxytocinase acts as a cystine-aminopeptidase ("CAP") cleaving the cystine-tyrosine bond of the oxytocin molecule. This observation led the authors to use l-cystine-di-β-naphthylamide ("CDNA") as an artificial substrate which resembles the cystine-peptide bond. Oxytocinase as a cystine-aminopeptidase hydrolyses CDNA. The released β-naphthylamine ("BNA") can be measured easily using the BRATTON-MARSHALL method (7) as described and modified by GREEN et al. (8). This chemical method has been used frequently for CAP determinations replacing rather time-consuming methods which are based on the bioassay of non-inactivated oxytocin (6, 9, 10). It is generally believed that CAP activity is due to oxytocinase. By means of this chemical method, CAP activity has been found to increase during pregnancy, and to reach highest values at term. CAP-activity decreases after delivery and drops within 6 weeks to levels of non-pregnant individuals.

Regardless of pregnancy, serum exhibits leucine-aminopeptidase ("LAP") activity, which is easily measured by the amount of BNA released by incubation of l-leucine-β-naphthylamide-hydrochloride ("LNA") with serum. There is a gradual increase of LAP activity during pregnancy, and at term it is found to be 3.5 times higher as compared to an non-pregnant state (11—13). Using starch gel electrophoresis, WINTERSBERGER and TUPPY (14) have separated two enzymes, CAP and LAP, from serum of pregnant women. — Small CAP-activity values are detectable in the non-pregnant state, and there is a thirteen-fold increase in enzyme activity from early pregnancy until term. CAP was found to be capable of hydrolysing LNA as well as CDNA (15), but no evidence has been presented yet on the specificity of LAP. In 1959, PORATH and FLODIN (16) introduced dextran gels under the trade name of "Sephadex" for separations of low molecular weight substances ($< 50{,}000$) and desalting operations according to differences in molecular size. With less extensively crosslinked gels FLODIN and KILLANDER (17) extended the range of possible separation towards molecules of larger size. The fractionation of human-serum proteins by gel filtration has been shown by the authors to be based mainly on the molecular sieve principle, indicating a convenient and mild procedure, which led to its application for serum cystine- and leucine aminopeptidase studies.

This report is concerned with separation of the two enzymes by gel filtration and a description of the hydrolytic activities of the two enzymes towards CDNA and LNA during the course of human pregnancy.

Material and Methods

Enzyme preparation

Assays for CDNA and LNA hydrolysis were conducted with three different preparations: a) dialyzed serum, b) serum subjected to gel filtration and c) highly purified oxytocinase preparations from retroplacental blood.

a) Blood as the source of enzymes was drawn from non-pregnant and pregnant women in early and late pregnancy, at term, during labor and postpartum. As soon as clotting was complete, serum was obtained by centrifugation. Only samples free of hemolysis were used. Serum was subsequently dialyzed against 0.2 *m* NaCl in 0.1 *m* triethanolamine-hydrochloride buffer pH = 8.0 at +3° overnight before it was assayed for CAP and LAP activity or subjected to gel filtration.

b) After dialysis serum was fractionated on "Sephadex G-200" gel according to the method of FLODIN and KILLENDER (17). The gel "Sephadex G-200"[1]) with a particle size of 140—400 mesh (U. S. sieve series), has been prepared in the following manner: the dry gel was allowed to swell over one week in 0.1 *m* triethanolamine-hydrochloride buffer pH = 8.0, containing 0.2 *m* NaCl, at +3°. Fines were removed frequently by suction and the gel was washed with additional buffer. Columns were packed by puring the thick slurry into the partially buffer filled column while the outlet was opened. This was done after a 2 cm layer of "Sephadex G-25" gel had been prepared to shield the fine pores of the filter glass plate. The column top surface was carefully leveled by rotation and protected with a well fitted filter paper. Buffer was run overnight to stabilize the bed of the gel. During this study three different column sizes were used giving bed volumes of 450, 550 and 570 m*l*. For each column the height to diameter ratio was approximately 10:1. The whole operation was done at +3°. With the aid of a 0.1 cm. i. d. polyethylene tubing connected to a syringe, serum samples were placed on the column as well as the first 50 m*l* of buffer for elution, after the serum had entered the gel. Elution was made with a comparatively low hydrostatic pressure of 20 to 30 cm buffer above the gel surface. The buffer was fed from an equal level reservoir with a large diameter connected to the column. Flow rates ranged from 20 to 30 m*l* per hour. The procedure was done conveniently overnight. Fractions of 5 to 7 m*l* were collected by use of an automatic fraction collector. Protein concentration was measured by means of optical density determination on diluted samples of each fraction. In some fractions, it was necessary to add a drop of 0.1 M NaOH to abolish turbidity. There was virtually no loss of protein during the procedure of gel filtration. From all fractions of a gel filtration assayed for CDNA and LNA hydrolyzing activity one could account for 80 to 95 % of the enzyme activity found in unfractionated serum. Enzyme activity after gel filtration was measured within 36 hours after completion of filtration. The fractions were kept at +3° until analysis.

c) Highly purified ocytocinase was prepared according to TUPPY and WINTERSBERGER (15). Retroplacental blood, which has a higher concentration of oxytocinase than venous blood, was collected from numerous deliveries over 8 to 14 day periods and immediately centrifuged. The serum was stored under refrigeration until 1000 m*l* were collected. After precipitation with ammonium sulfate (40—60 % saturation) and subsequently with "Rivanol" (6,9-diamino-2-ethoxyacridine-lactate)[2]), the enzyme solution was freed from inactive proteins and "Rivanol" by Bentonite, which then was removed by centrifugation. These enzyme preparations were finally chromatographed on hydroxylapaptite [$Ca_5(PO_4)_3OH$] columns which have been prepared according to TISELIUS, HJERTEN and LEVIN (18). Elution of oxytocinase from these columns of approximately 500 m*l* bed volume and a height to diameter ratio of about 11:1 was achieved with 0.01 *m* thriethanolamine-hydrochloride buffer pH = 7.4. The whole procedure of purification was done at +3°. The degree of purification is listed in table 1. The preparation suspended in complete Freund adjuvant was used for injection into a rabbit. An antiserum was obtained, which yielded together with the antigen a single line of precipitation when the double-diffusion-in-gel technique was used. These findings agree with those of TUPPY and WINTERSBERGER.

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Tab. 1
Purification of CAP (oxytocinase) from retroplacental serum

Step	Volume (m*l*)	mg protein/ m*l* solution	mg BNA freed per gm of protein/hour*)	yield
Retroplacental serum	1000	66.3	0.65	100%
Ammonium sulfate precipitation	720	20.6	1.76	61%
"Rivanol" precip. and Bentonite treatment	54	5.36	14	9.5%
Chromatography on $Ca_5(PO_4)_3OH$	22.5	0.02	3380	3.6%

*) BNA = naphthylamine, freed by hydrolysis from DCNA.

Substrates

L-leucyl-β-naphthylamide HCl (LNA), (lots 501812 and 504317)[5]) and l-cystine-di-β-naphthylamide (CDNA), (lot 502317)) and used for determination of LAP and CAP activity. Stock solutions of LNA (0.01 *m*) were prepared in dist. water. CDNA (0.005 *m*) was dissolved in 0.01 *n* HCl. The substrates were refrigerated and used within 14 days of preparation of stock solutions.

Incubation

Enzyme activity has been determined by incubation at + 37° for 1, 2 and 4 hours, respectively. Appropriate aliquots of different enzyme preparations were incubated with substrate solutions at a final concentration of 2.2×10^{-4} *m* LNA or 1.1×10^{-4} *m* CDNA. A 0.1 *m* triethanolamine-hydrochloride buffer pH = 7.4 was used. This buffer contained 6 g per 100 m*l* of bovine serum albumin (Cohn fraction V)[1]) which was used to prevent precipitation of CDNA from solutions of low protein concentrations (19). Enzymatic reactions were terminated by addition of trichloracetic acid, reagent grade, in amounts to yield a final concentration of 5% (w/v).

Determination of BNA freed by enzymatic hydrolysis

After centrifugation of the incubation mixture to which trichloracetic acid had been added, 1.0 m*l* of clear supernatant was transferred into a 50 m*l* test tube containing 9.0 m*l* of a mixture of 2 parts 0.36 *n* HCl and 1 part acetone. The following steps were performed in a dark room under red light while the test tubes were kept at + 37° and repeatedly shaken: 1.0 m*l* of a 0.1% $NaNO_2$ solution, 1.0 m*l* of a 0.5% ammonium sulfamate solution and 1.0 m*l* of a 0.1% aqueous solution of N-(1-naphthyl)-ethylene-diamine-di-hydrochloride[2]) were added in three minute intervals.
The blue violet diazo color developed over the next three hours in darkness. After 3 hours, absorption was determined in a Beckman Model DU Spectrophotometer at 565 mμ using 1.0 cm pathway cuvettes and blanks derived from identical incubation mixtures to which trichloracetic acid was added at once in order to prevent hydrolysis. Absorbance proved to be directly proportional to the amount of BNA liberated by enzymic hydrolysis. A series of calibration experiments revealed an absorption of 0.242 ± 0.004 S. E. (n = 18) for 10 μg BNA per 1.0 m*l* of supernatant.
Two determinations of LAP- or CAP-activity in a single sample did not differ more than 3% from each other. Enzyme activity has been calculated according to Tuppy and Nesvadba (6) given in mg BNA freed per hour from LNA or CDNA by 100 m*l* of serum. The amount of BNA has been calculated for 100 m*l* of serum and 1 hour incubation time at 37° and pH = 7.4, using 0.1 *m* triethanolamine-hydrochloride buffer.

Results

Leucine aminopeptidase- and cystine-aminopeptidase-activity was determined in sera of non-pregnant and pregnant women, without previous fractionation of serum proteins. LAP- and CAP-activity was shown to increase as pregnancy proceeded towards term. Levels at term were found to be 3.5 and 12.5 times higher, respectively, than in the non-pregnant state. LAP- and CAP-activity in the sixth and tenth weeks of gestation did not differ from that in non-pregnancy. The results which were obtained with unfractionated sera are summarized in table 2.

Tab. 2

Unfractionated serum from	mg BNA + freed by enzymatic hydrolysis from CDNA	LNA
non-pregnant women	0.49 ± 0.05 s. e. (n = 5)	17.9 ± 3.5 s. e. (n = 5)
6*th* week of gestat.	0.47	17.7
10*th* week of gestat.	0.44	16.8
18*th* week of gestat.	1.69	23.2
at term	6.16 ± 0.35 s. e. (n = 4)	66.3 ± 6.2 s. e. (n = 4)
during labor	6.75	59.1
2 days after deliv.	2.79	25.4

All sera were fractionated using gel filtration on "Sephadex G-200". The pattern of protein concentration in the eluate fractions exhibited three incompletely separated peaks, which have been shown by Flodin and Killander (17) to correspond with the α_2- and β_2-macroglobulins, the γ-globulins and the albumins, respectively. Maximum protein concentration in each of the three peaks was observed at constant elution volumes, as is indicated in table 3.
LAP-activity peaks were found in all sera regardless of whether the patient was pregnant or not. In pregnancy, a second peak of LAP-adtivity appeared which was

[1]) California Corporation for Biochemical Research, Los Angeles 63.
[2]) Eastman Kodak.

incompletely separated from the LAP-activity peak in non-pregnant sera (Fig. 1). This second peak decreased after delivery. Corresponding to the 'pregnancy LAP-activity peak', a peak of CAP activity was found in the same fractions. This peak increased during pregnancy and decreased after delivery. There was no CAP-activity peak nor second LAP-peak in serum fractions of the sixth and tenth week of gestation. For each column used the elution colume of LAP- and CAP peaks was observed to be constant (Tab. 3).

Tab. 3

No. of serum sample	Serum from:	volume of eluate in m*l* for maximum of protein concentration peak No. 1	2	3	enzyme activity LAP	LAP activity	CAP
column no. 1 volume = 540 ml							
1	pregnancy	160	214	279	222	183	183
2	non-pregnancy	160	216	281	221		
3	pregnancy	159	214	284	220	181	181
column no. 2 gel volume = 550 ml							
4	pregnancy	190	283	362	288	225	225
5	pregnancy	191	285	362	289	227	227
6	non-pregnancy	195	275	363	278		
7	serum of no. 6 + purified CAP	190	273	370	282	217	217
column no. 3 gel volume = 570 ml							
8	pregnancy	208	284	362	287	234	234
9	pregnancy	200	290	375	290	227	227
10	pregnancy	210	296	374	299	227	227
11	pregnancy*)	188	280	360	280		
12	non-pregnancy	196	287	364	287		

*) 6*th* week of gestation.

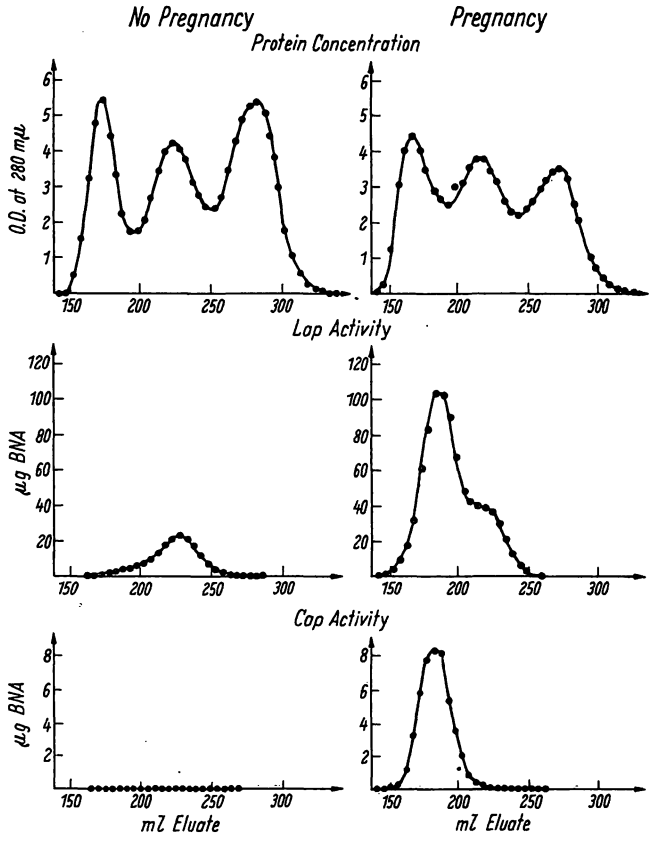

Fig. 1

Serum of a pregnant (term) and a non-pregnant woman has been subjected to gel filtration on "Sephadex G-200". Fractions have been assayed for protein concentration and LAP- and CAP-activity.

Since gel filtration dilutes serum fractions to such an extent that small CAP-activity (as it is present in the first half of gestation) cannot be assayed with accuracy, several fractions were pooled after determination of

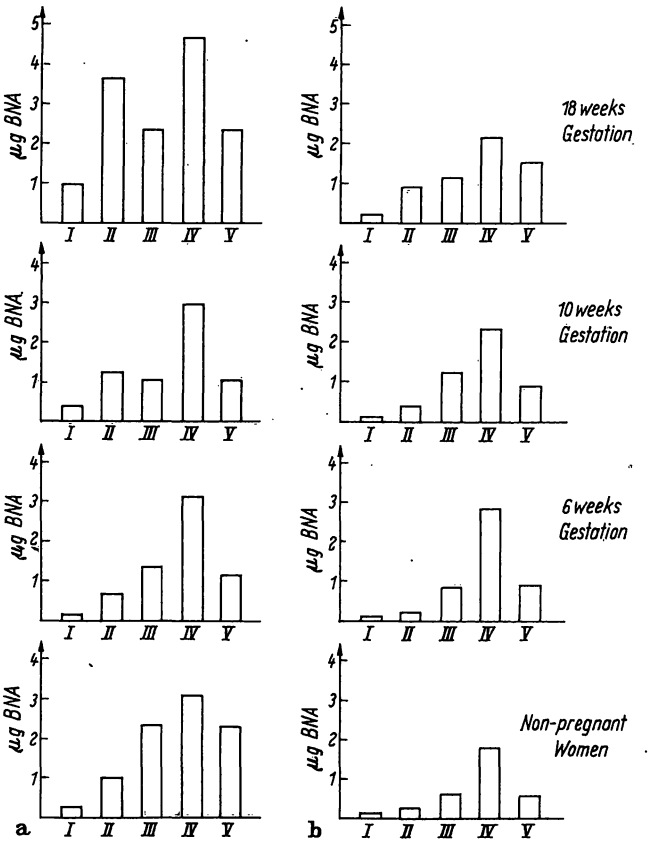

Fig. 2

10 m*l* of serum obtained from a non-pregnant woman and from three pregnant women were fractionated on Sephadex-G 200 gel. Aliquots of fractions were assayed for protein concentration and CAP- and LAP-activity.

Then aliquots of fractions were pooled in order to obtain 5 pools with II and IV containing peaks of CAP and LAP respectively. These pooled fractions were dialysed against distilled water, concentrated by ultrafiltration through collodium membranes and assayed for CAP- and LAP-activity. Results are expressed as µg BNA released from CDNA (A) and from LNA (B). BNA values for B have been calculated for enzyme concentrations and incubation times used for CAP-activity determinations (A) for demonstration of the quantitative relationship.

protein concentration, CAP- and LAP-activity. A total of five pools were taken from each fractionated serum, combining aliquots of 5 or 6 single fractions in each pool of 15 m*l* volume. Pool II and IV have been taken from the CAP-peak and LAP-peak, respectively, so that in addition to an intermediate pool between CAP and LAP, the first and the last pool also could be checked for CAP- and LAP-activity. The pooled fractions were dialyzed against distilled water overnight, at + 3°, and subsequently concentrated by ultrafiltration through collodium bags[1]), before being analyzed for CAP- and LAP-activity. Results from 4 sera were examined in this manner (one non-pregnant woman, three women in the sixth, tenth and eighteenth week of gestation), and are presented in figure 2. Only the sample from the eighteenth week of gestation presents significant evidence for CAP, whereas between the tenth week and sixth week as well as the non-pregnant state no significant difference could be found.

These experiments, which combine fractionation of serum on Sephadex gel and concentration of pooled fractions by ultrafiltration, reveal that LAP also hydrolyzes CDNA, which has been thought to be a substrate for CAP. Enzymatic hydrolysis of l-leucine-β-naphthylamide-hydrochloride by LAP is 74 ± 12 times faster than hydrolysis of l-cystine-di-β-naphthylamide, as has been calculated from results shown in figure 2, when 0.1 *m* triethanolamine-hydrochloride buffer pH = 7.4 was used. Highly purified CAP has been confirmed to hydrolyse LNA 11 times faster than CDNA.

Comment

Values of leucine-aminopeptidase activity have been found in sera of pregnant and non-pregnant women. An increase of LAP-activity during pregnancy was noted, and at term values were found to be 3.5 times higher as compared to non-pregnant levels (6, 9—12). Also, cystine-amino-peptidase activity (CAP) has been described in non-pregnant women. As pregnancy proceeds to term, values are approximately 13 times higher than in non-pregnant women (6, 9, 10). — Wintersberger and Tuppy (14) separated CAP and LAP in serum of pregnant women by starch gel electrophoresis using a histochemical staining procedure for detection of enzyme activity. In non-pregnant patients, only one single zone of LAP activity was found. An additional band of LAP activity appeared in pregnancy and was demonstrated in the second half of gestation. Page et al. (20) confirmed these findings and, in addition, using vertical starch gel electrophoresis, found a second CAP band exhibiting LAP and CAP activity in late pregnancy.

In the present study, it is shown that the enzyme which appeared in pregnancy displaying LAP and CAP activities is distinguishable from LAP by gel filtration on "Sephadex G-200". Assuming the molecular sieve principle to be responsible for the difference of distribution coefficients (0.089 for CAP and 0.26 for LAP), it appears to be conclusive that CAP has a greater molecular weight than LAP. — CAP purified by precipitation procedures and chromatography on hydroxylapatite hydrolyzes LNA 11 times faster than CDNA. This is in agreement with values reported by Tuppy and Wintersberger (15). That the 3.5 fold increase in LAP activity (table 2) found at term is caused by the appearance of CAP is clearly shown in figure 1. It is also demonstrated in this study that LAP not only hydrolyzes LNA, but also CDNA. The lack of substrate specificity could account for the discrepancy between the thirteenfold increase of serum CAP activity during pregnancy, and the at least hundred-fold increase in oxytocinase activity found with bioassay methods. LAP hydrolyzes CDNA at a slow rate, which however, produces "pseudo-CAP values" in serum of non-pregnant women. Subtraction of CAP activity which is due to LAP will reduce net CAP-activity in early pregnancy to such small levels that towards term one can expect an increase of CAP-activity which is comparable to ratios found with bioassay methods. The ability of LAP to hydrolyze CDNA shows that this substrate cannot be used to unequivocally demonstrate the presence of CAP-activity, which might be considered for the evaluation of histochemical studies on oxytocinase (21).

There is no indication from this study that yearly pregnancy can be detected by an increase in either CAP- or LAP-activity in the first trimester. A significant increase can be found at mid-pregnancy only. However, further determinations would be required whether this is statistically significant. — The chemical oxytocinase determination has its limitation because oxytocinase has a powerful LAP-activity and LAP present in serum and body tissues hydrolyzes cystinylpeptides, too. When CAP-activity due to oxytocinase equals CAP activity due to LAP during the second trimester of pregnancy, LAP and CAP determinations cannot yield reliable information about serum oxytocinase activity.

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Polarographische Untersuchungen der elektrophoretisch aufgetrennten Albuminfraktion

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Eine von HOMOLKA angegebene kombinierte elektrophoretisch-polarographische Untersuchung der Serumproteine wurde modifiziert. Es wurden 14 gesunde Versuchspersonen und 36 Patienten mit verschiedenen Krankheitsbildern untersucht und das Ergebnis mit dem der Papierelektrophorese verglichen. Es ergab sich keine höhere Empfindlichkeit für das Vorliegen entzündlicher Prozesse gegenüber der üblichen Papierelektrophorese. Es ließen sich qualitativ bedingte Denaturierungsveränderungen in der elektrophoretisch aufgetrennten Albuminfraktion nachweisen. Die Ursachen werden diskutiert, wobei auf die mögliche Bedeutung der Mukoproteine hingewiesen wird.

A combined electrophoretic-polarographic study of serum proteins, described by HOMOLKA, was modified. 14 healthy persons and 36 patients with various illnesses were studied and the results compared with those obtained by paper electrophoresis. It was no more sensitive in the detection of inflammatory conditions than the normal paper electrophoresis. Qualitative denaturation changes were found in the electrophoretically separated albumin fraction. The reasons for this are discussed and it is concluded that the mucoproteins may be responsible.

Die Bestimmung der Aktivität entzündlicher Prozesse ist ein dringendes Problem, das sowohl für die Frühdiagnose als auch für die Therapie zunehmend an Bedeutung gewinnt. Gesucht werden hochempfindliche Methoden, die klinisch und labormäßig weitgehend stumm verlaufende Prozesse anzeigen können. Solche Indikatoren spielen auf dem Gebiete der rheumatischen Erkrankungen eine Rolle, doch sind sie auf anderen Gebieten der Medizin von nicht zu unterschätzender Bedeutung. — Unter diesem Gesichtspunkt wurde eine von HOMOLKA (1) angegebene kombinierte elektrophoretisch-polarographische Quantitäts- und Qualitätsbestimmung der Serumeiweißfraktionen, die empfindlicher als die übliche Papierelektrophorese sein sollte, geprüft.

Methodik

Prinzip

Die Serumeiweiße werden in der Papierelektrophorese aufgetrennt und die einzelnen Fraktionen nach ihrer Elution auf ihre Denaturierbarkeit polarographisch untersucht, wobei die aufschlußreichsten Veränderungen sich in der Albumin-Fraktion finden, die deshalb auch allein weiter verfolgt werden soll. Mit dieser Methode werden sowohl qualitative als auch quantitative Veränderungen der einzelnen Eiweißfraktionen summarisch erfaßt. Es ist bekannt (2), und es läßt sich im Versuch leicht zeigen, daß polarographische Aktivität der einzelnen Serumeiweißfraktionen nicht nur von ihrer Qualität, sondern auch von ihrer Quantität abhängig ist, wobei besonders zu beachten ist, daß die Freisetzung nach Alkaligabe *nicht* der Eiweißmenge linear proportional erfolgt. In dem hier interessierenden Bereich zwischen 20 und 40 μg Albumin steigt die Freisetzung mit zunehmender Menge deutlich stärker an. Bei 50 μg wird ein Maximum an Freisetzung erreicht. Größere Eiweißmengen führen wieder zu einer relativen Verminderung der Freisetzung.

Wenn in der elektrophoretisch aufgetrennten Albuminfraktion eine erniedrigte Freisetzung polarographisch aktiver Gruppen nach Alkalidenaturierung gefunden wird, dann kann das sowohl durch eine Verminderung der Eiweißmenge als auch durch qualitative Veränderungen der im Albuminbereich gelegenen polarographisch wirksamen Gruppen bedingt sein. Eine Differenzierung ist nicht möglich. Es interessierte zunächst, ob und in welchem Umfang zusätzliche qualitative Änderungen in der elektrophoretisch aufgetrennten Albuminfraktion bei entzündlichen Prozessen sich nachweisen ließen. Aus diesem Grunde wurden die nach papierelektrophoretischer Auftrennung erhaltenen Proteinfraktionen vor dem Polarographieren jeweils auf einen