Table III. Quantitative determinations of DNA

DNA in the liver (mg/g)	Rats		
	2 months (not treated)	15 months (not treated)	26 months (not treated)
Extraction following SWINDLEHURST[11]	2.042 ± 0.136	1.403 ± 0.110	1.126 ± 0.094
Extraction following SCHNEIDER[12]	2.368 ± 0.181	2.213 ± 0.174	1.976 ± 0.120

The mean values are reported ± the average quadratic error of the mean.

such values are not comparable with those found in young rats, considering the high level of alkaline phosphatase present in youngs.

DNA and RNA contents of the liver and radioactivity incorporated. The findings reported in Table II show that the aqueous thymus extract causes, in the old rats as compared to the controls, a considerable and significant increase in the content of DNA extracted in the polymerized state with sodium lauryl sulfate according to the SWINDLEHURST method. No significant variation was found for RNA. This fact has also been confirmed by a different method of nucleic acids extraction in NaCl (data not reported). It should be kept in mind that the ratio body weight/liver weight in treated as well as untreated animals was statistically unchanged.

As for the incorporation of H^3-uridine, the data reported in Table II demonstrate that the aqueous thymus extract causes a considerable decrease of radioactivity incorporated into DNA and an insignificant decrease of radioactivity incorporated into RNA. It is interesting to note that the values of incorporation into DNA demonstrate a detectable activity of enzymatic reduction of the ribonucleotides in the liver of old animals. The data regarding DNA radioactivity can be correlated to the results obtained by PRICE et al.[17], according to which the template activity of DNA for DNA polymerase increases with age; this is interpreted by the authors as an accumulation of defective chains of DNA with aging, as demonstrated also by PELC[18].

In a subsequent experiment, we verified whether the increase of the polymerized DNA extract, found in the liver of old rats treated with the thymus extract as compared to the controls, could be attributed to a real increase in the total DNA content of the liver or to a different physical-chemical state of the deoxyribonucleoproteins, with consequent different extractability. In this respect, we compared the results of the extraction of DNA from the liver of 2-, 15- and 26-month-old animals, carried out using the methods of SWINDLEHURST and SCHNEIDER (Table III). From the results reported in Table III one can see that there is a considerable, progressive and significant decrease in the content of extractable hepatic DNA at the polymerized state from 2 to 15 months and from 15 to 26 months of age; vice versa the content of hepatic DNA extracted by the SCHNEIDER's method, which entails depolymerization, presents a slight decrease with aging.

The comparison of data reported in Tables II and III demonstrates that the action exerted by the aqueous thymus extract on hepatic DNA is to be referred to an increase of extractability and not to the total nucleic acid content of the liver. This regulatory activity is probably related to modifications of interactions in the DNA-protein complex.

Riassunto. La somministrazione di estratti acquosi di timo provoca nei ratti vecchi (26 mesi) una diminuzione della concentrazione delle proteine e del colesterolo del siero, e un aumento della concentrazione ematica dell'ATP. L'estratto provoca inoltre nel fegato un aumento del DNA estraibile allo stato polimerizzato, e una diminuzione dell'incorporazione di uridina H^3 nel DNA.

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Binding of Tryptophan to Different Serum Proteins in Humans and in Rats

L-tryptophan is the only amino acid present in plasma which is bound to proteins. Studies of MCMENAMY et al.[1] and MCMENAMY and ONCLEY[2] have shown that albumine is the only plasma protein which binds tryptophan appreciably. L-tryptophan was shown to bind at one site of the albumine molecule in a highly stereospecific manner. However, since these authors have analyzed only a few serum protein fractions, their data do not exclude that L-tryptophan might combine with other serum proteins in addition to albumine. This possibility has been investigated in the present study. This investigation seemed to us of considerable interest also in the light of recent findings that unbound serum tryptophan controls brain tryptophan level and serotonin synthesis[3-6] and that different drugs are capable of displacing serum tryptophan from its protein binding[7,8] and of increasing brain tryptophan and serotonin turnover[4,5,8].

Material and methods. Chemicals. C^{14}-L-tryptophan (45 mCi/mmole) and C^{14}-L-Leucine (10 mCi/mmole) were obtained from Amersham. C^{14}-L-tryptophan was purified before use by Dowex-X4 column chromatography as described by COSTA et al.[9].

Human albumine (purity 100%), prealbumine (purity 99%) and γ-globuline (purity 99%), obtained from

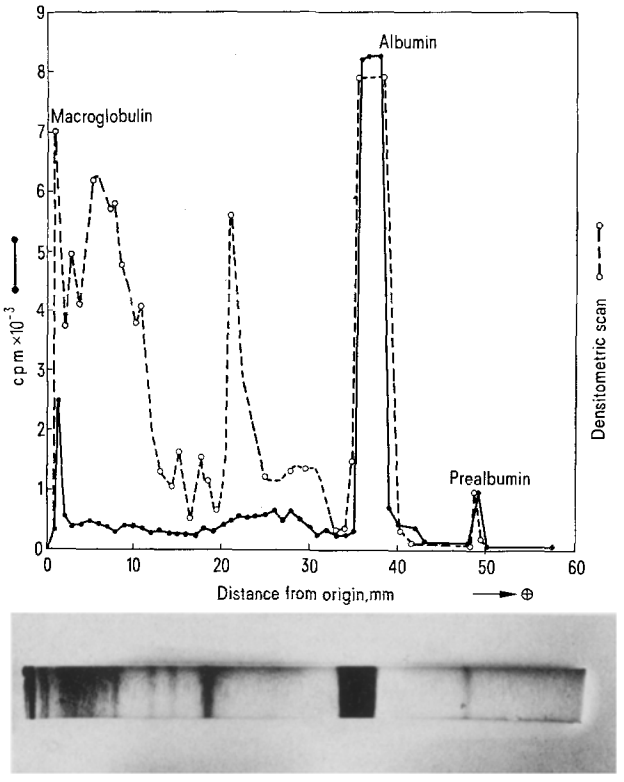

Fig. 1. The solid line represents the distribution of C^{14}-L-tryptophan in electrophoretic pattern of human serum, when a trace amount of C^{14}-L-tryptophan was incubated with serum for 60 min at 37 °C before electrophoresis. The dotted line represents the densitometric scan obtained in a Joyce Loebl densitometer (Chromoscan). Gels were stained with Amido black, photografed, and then cut in 1 mm segments, each of which was counted for radioactivity.

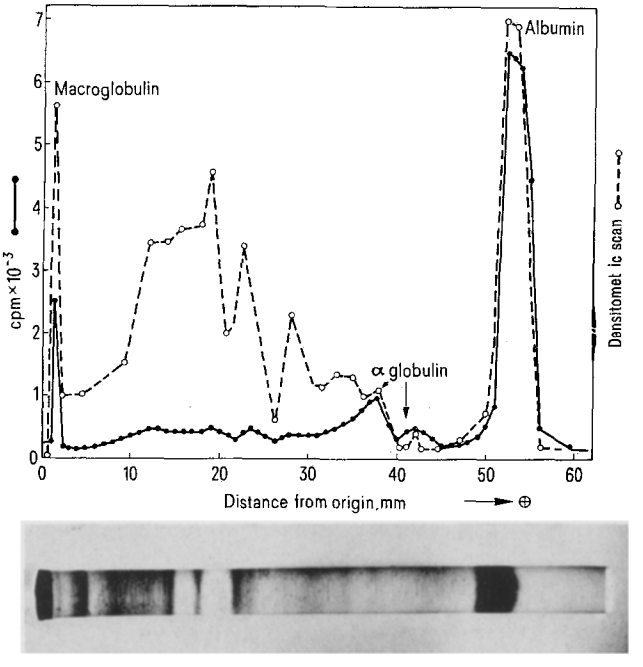

Fig. 2. The solid line represents the distribution of C^{14}-L-tryptophan in electrophoretic pattern of rat serum, when a trace amount of C^{14}-L-tryptophan was incubated with serum for 60 min at 37 °C before electrophoresis. The dotted line represents the densitometric scan obtained in a Joyce Loebl densitometer (Chromoscan). Gels were stained with Amido black, photographed, and cut in 1 mm segments, each of which was counted for radioactivity.

Hoechst, were dialyzed before use for 12–24 h at 5 °C against bi-distilled water.

Electrophoretic separation of serum proteins. C^{14}-L-tryptophan (5–10 μCi) was incubated for 60 min at 37 °C with pooled sera (2 ml) obtained from either healthy young male subjects, or from male Wistar rats, weighing 250–280 g. The various serum proteins were separated by polyacrylamide electrophoresis as described by ORNSTEIN[10]. To determine the radioactivity present in the various protein fractions, gel columns were stained for 1 h in a solution of 0.1% amido-black in methanol, acetic acid and water (4:1:5). Stained gels were sectioned into 1 mm thick slices, each of the slices was dissolved in 0.5 ml H_2O_2 (31%) at 50 °C for 5 h in a liquid scintillation vial to which 10 ml of Insta-Gel scintillation fluid were added. The radioactivity was measured in a Tri-Carb liquid scintillation counter.

Results. Figures 1 and 2 show the radioactivity superimposed on the densitometric scans of the gels after staining. In human serum, 3 peaks of radioactivity were localized in the albumine, prealbumine and macroglobulin fractions, respectively.

In rat serum, the radioactivity accumulated not only in the albumine fraction but also in the α-globulin zone and macroglobulin fraction.

The binding of tryptophan to human albumine and prealbumine was studied by incubating C^{14}-L-tryptophan with either proteins for 60 min at 37 °C and then centrifuging the samples in visking dialysis tubings as previously described[5]. The radioactivity present in the undialyzed samples and in the dialysates was measured.

As the Table shows, albumine bound 13%, 44% and 62% of the tryptophan present when the molar ratio of albumine to tryptophan in the medium was 1:1, 5:1 and 10:1, respectively. The prealbumine bound 14% of the tryptophan present at a molar ratio of prealbumine to tryptophan of 1:1. Due to the high cost of the material, we could not carry out studies with higher concentrations of prealbumine.

Finally, no binding occurred between C^{14}-L-leucine and albumine and between γ-globulin and C^{14}-L-tryptophan.

Discussion. The present work has confirmed previous studies showing that L-tryptophan binds to serum albumine. Moreover, the results of the electrophoretic study have shown that this amino acid binds, both in human and rat serum, also to other proteins.

In human serum, C^{14}-L-tryptophan was localized in the albumine, prealbumine and macroglobulin fractions. In the rat serum, C^{14}-L-tryptophan was localized in the albumine, α-globuline zone and macroglobulin fractions.

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Binding of C^{14}-L-tryptophan by human serum albumine and prealbumine

Protein	Concentration	Amino acid [a]	dpm in the undialyzed sample	dpm in the dialysate (m*M*)
Albumine	1.4×10^{-3}	C^{14}-L-tryptophan	5293	4580
Albumine	7.0×10^{-3}	C^{14}-L-tryptophan	5468	3062
Albumine	1.4×10^{-2}	C^{14}-L-tryptophan	5814	2252
Prealbumine	1.4×10^{-3}	C^{14}-L-tryptophan	5625	4856
γ-Globulin	1.4×10^{-3}	C^{14}-L-tryptophan	5794	5772
Albumine	1.4×10^{-3}	C^{14}-L-leucine	5559	5547

Each value is the average (and range) of 3 experiments. [a] C^{14}-L-tryptophan or C^{14}-L-leucine were added at a molar concentration of 1.4×10^{-3} with a specific activity of 50 μCi/m*M*.

The results of the ultrafiltration dialysis with purified human albumine and prealbumine have confirmed that L-tryptophan binds to both albumine and prealbumine fractions and have indicated that it has the same affinity for either fraction.

Since albumine is present in plasma at a much higher concentration than the other proteins, the results indicate that most of the tryptophan present in plasma is bound to albumine and suggest that the significance of the binding of tryptophan to the other protein fractions is limited under physiological conditions.

Riassunto. Sieri di uomo e di ratto sono stati incubati con C^{14}-L-triptofano e sottoposti a elettroforesi su gel di poliacrilamide. Nel siero umano la radioattività si è localizzata nelle frazioni albuminica, prealbuminica e macroglobulinica. Nel siero di ratto il C^{14}-L-triptofano si è localizzato nella zona delle albumine, α-globuline e macroglobuline.

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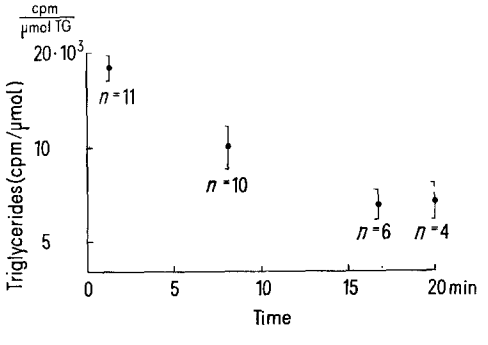
Studies on the Turn-Over of Plasma Triglycerides using Triglyceride ^{14}C-labelled Chyle in Pregnant Rats

Several animal species, including man and the rat, display a hypertriglyceridaemia during the last week of pregnancy[1]. The rise in plasma triglycerides could result either from a rise in the output, or from a fall in the rate of removal from circulation. Some evidence for the latter hypothesis was given by Otway[2] and Hamosh[3], because they noted a reduction in the activity of the plasma-clearing lipase after the 19th day of pregnancy.



The specific activity of plasma triglycerides as a function of time after the i.v. injection of ^{14}C-1-palmitate labelled chyle. At zero time, 1×10^6 counts/min (recovered in the triglycerides of the injected chyle) per 300 g body weight were injected. Data are mean values $\pm$ standard errors for several estimations (The number of estimations is indicated by *n*). The turn-over time was calculated by regression analysis to be 17.3 $\pm$ 2.7 min.

The purpose of the present study was to evaluate the rate of plasma triglyceride removal from the circulation on the 21st day of pregnancy.

Methods. A thoracic duct cannulation had been performed on male Wistar rats according to the method of Bollman[4]. The rats were then given 100–200 μC of ^{14}C-1-palmitate dissolved in 1 ml corn oil. The chyle was collected for 12 h in a flask containing citrate. The chyle was concentrated by centrifugation for 60 min at 70,000 $\times g$. The top layer was aspirated and resuspended in 0.9% NaCl. The chyle was kept at 4 °C. All injection procedures were completed within 10 days after collection of chyle. The chyle was injected into the tail vein of pregnant Wistar rats (21 days) weighing 320 $\pm$ 10 g. At appropriate times, they were anesthetized with ether and blood drawn from the vena cava inferior. The blood and an aliquot of the injected chyle were extracted for lipids according to the method of Folch.[5] The triglycerides were solated by thin layer chromatography and assayed for quantity and radioactivity, and the specific activity was calculated. The disappearance rate of labelled chyle was calculated by linear regression analysis. The plasma volume of pregnant rats was measured by dye dilution technique, according to Hamilton[6]. Details of the methods used have already been described[7].

Results. All the values given were normalized to a constant injected dose (1×10^6 counts/min) of radioactivity (recovered in the triglyceride fraction of the chyle), and a body weight of 300 g. The Figure represents the specific activities of plasma triglycerides as a function of