and alkaline phosphatase activities were determined as described previously[5,7].

Results. The basal activity of NADH-cyt. c reductase in dog renal cortex microsomes is about 100 nmoles/mg protein/min and is approximately doubled in the presence of 100 m*M* sodium ions. The stimulation of the enzyme in the presence of different NaCl concentrations is illustrated in Figure 1 where it can be observed that maximal stimulation is attained at a concentration of 75 m*M*. High concentrations of sodium become inhibitory. A similar stimulation is encountered when sodium chloride is replaced with sodium sulphate. Potassium ions have the same effect as sodium, the stimulation by choline chloride is less than that observed with sodium or potassium, but mannitol of the same tonicity has no effect. Magnesium ions, surprisingly, are inhibitory.

Studies with inhibitors, and the electrophoretic data presented below, suggest that at least 2 different enzyme systems are present in this preparation, one which is stimulated by sodium and one which is not. Rotenone only inhibits the enzyme that is insensitive to sodium. Antimycin affects neither enzyme, nor does ouabain. Ethacrynic acid (1 m*M*) had no consistent effect.

The presence of a microsomal Na^+-stimulated NADH-cyt. c reductase appears widespread, since an enzyme with essentially identical properties was located in the kidneys of dogs, rats and guinea-pigs, in the guinea-pig and dog small intestine, and in the dog colonic mucosa. Its presence in the rat liver has been described previously[3].

Various attempts at defining the cytological location of the enzyme were carried out. First, as seen in the Table, it occurs almost exclusively in the microsomal fraction, whereas the Na^+-insensitive homologue occurs both in the nuclear-mitochondrial and the microsomal fractions. Na^+-K^+-ATPase preparations have been shown to be enriched in the presence of deoxycholate and on ageing for 48 h at 0 °C in the presence of the detergent, due probably to opening of closed vesicles[5]. Deoxycholate eliminated all activity of the Na^+-dependent NADH-cyt. c reductase from the nuclear-mitochondrial fraction, though Na^+-K^+-ATPase activity was concomitantly increased in this fraction. Thus, taken together, these findings suggest that the reductase is not located within the same membrane as the Na^+-K^+-ATPase.

Sophisticated separation techniques (free-flow electrophoresis) were applied to crude membrane fractions from rat kidney cortex, and the activity of the enzyme, as well as that of other marker enzymes, was determined in the fractions eluting from the electrophoresis (Figure 2). It was found that the Na^+-stimulated NADH-cyt. c reductase did not follow either the elution pattern of Na^+-K^+-ATPase or that of alkaline phosphatase. Secondly, there was a notable difference in elution pattern of the Na^+-stimulated activity from that of the Na^+-insensitive NADH-cyt. c reductase.

Discussion. The results disclosed in the present survey would appear to preclude the possibility that the Na^+-stimulated NADH-cyt. c reductase activity in microsomal fractions might be involved in membrane sodium transport, and to render unnecessary in the present context a more profound study of the enzyme. Thus it does not seem to be located in either of the plasma membranes of the proximal tubular cell, its stimulation by sodium is not specific for that ion, and it is not affected by ethacrynic acid, which is apparently a specific inhibitor of the ouabain-insensitive sodium pump in this tissue[1,2]. Thus the enzyme fails to fulfil three of the vital requirements for identification with sodium-pumping activity.

The biochemical role of this enzyme (which was discussed previously by SIEKEVITZ[3]) has not been clarified by the present investigations. It is known that the microsomal cytochrome reductases are extremely complex[8-11]. However, it seems that at least the Na^+-stimulated fraction is not bound to either plasma membrane. The possibility remains that it is identical with the rotenone-insensitive, antimycin-insensitive NADH-cyt. c reductase of the golgi apparatus described by FLEISCHER at al.[12] who unfortunately did not test the ion sensitivity of their enzyme[13].

Résumé. En présence de sodium, l'activité totale de la NADH-cytochrome c réductase rénale était doublée; le potassium induit le même effet stimulateur. Cette enzyme sensible aux cations n'est inhibée ni par la roténone ni par l'antimycine. Elle était également insensible à l'ouabaïne et à l'acide éthacrynique. Des expériences destinées à révéler sa localisation cytologique n'ont que partiellement réussi: l'enzyme ne semble pas être associée a la membrane de la bordure en brosse, ni à la membrane péritubulaire. Ces résultats suggèrent qu'elle n'est pas impliquée dans les mécanismes des pompes à sodium.

A.-L. LUISIER, J.W.L. ROBINSON and R. KINNE

Département de Chirurgie Expérimentale, Hôpital Cantonal Universitaire, CH-1011 Lausanne (Switzerland); and Max-Planck Institut für Biophysik, Kennedy Allee 70, D-6 Frankfurt-am-Main (Federal Republic Germany),
21 January 1974.

[7] R. KINNE, J.-E. SCHMITZ and E. KINNE-SAFFRAN, Pflügers Arch. Eur. J. Physiol. *329*, 191 (1971).
[8] H.-U. SCHULZE, H. GALLENKAMP and HJ. STAUDINGER, Hoppe-Seyler's Z. physiol. Chem. *351*, 809 (1970).
[9] T. HARA and S. MINAKAMI, J. Biochem. *69*, 317 (1971).
[10] K. MIHARA and R. SATO, J. Biochem. *71*, 725 (1972).
[11] K. STAROŃ and Z. KANIUGA, Hoppe-Seyler's Z. physiol. Chem. *353*, 14 (1972).
[12] B. FLEISCHER, S. FLEISCHER and H. OZAWA, J. Cell Biol. *43*, 59 (1969).
[13] This work was supported by the Fonds National Suisse and the Deutsche Forschungsgemeinschaft. We are grateful to Mlles H. ASPER and D. METTRAUX in Lausanne and Frau R. PLATE in Frankfurt for their excellent technical assistance.

Young and Old Rats. ATP, Alkaline Phosphatase, Cholesterol and Protein Levels in the Blood; DNA and RNA Contents of the Liver. Regulation by an Aqueous Thymus Extract

Various authors have attributed to the thymus an important role in the physiology of development[1]. In addition, since the involution of the thymus also entails a general decline of immunocompetence, and different authors[2-4] have suggested that aging could depend on an immunological disorder, PANTALOURIS[5] has postulated the hypothesis of a direct correlation between the thymus and senescence.

Table I. Concentrations of ATP, cholesterol, total proteins, and activity of alkaline phosphatase

	Rats		
	2 months (not treated)	26 months (not treated)	26 months (treated)
ATP in the blood (mg/100 ml)	31.20 ± 1.70	24.67 ± 1.86	29.28 ± 1.20
Cholesterol in the serum (mg/100 ml)	80.00 ± 11.00	180.00 ± 46.00	93.00 ± 36.00
Serum Proteins (g/100 ml)	6.25 ± 0.55	9.54 ± 1.05	7.37 ± 0.58
Alkaline phosphatase in the serum (nm/ml)	228.00 ± 22.00	140.00 ± 53.00	32.10 ± 6.63

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

These observations have led us to study the action of an aqueous thymus extract on a few hematic parameters (ATP, cholesterol, protein levels and alkaline phosphatase) and on the nucleic acid contents of the liver of old rats. In addition, the incorporation of H^3-uridine in the nucleic acids of the liver has been studied. The biological activity of this aqueous thymus extract was previously studied by us on cultures of *E. coli*[6,7]; it was observed that the extract exerts a regulatory activity on the synthesis of the nucleic acids.

Material and methods. For the preparation of aqueous thymus extract, the gland was carefully sectioned and the medullary portion removed by scraping was homogenized in the Potter-Helvehyem for 2 min with addition of 3 volumes of distilled water. The preparation was left to stand for 24 h, centrifuged at 20,000 *g* for 20 min and the supernatant collected. The supernatant was ultrafiltered under nitrogen 7 atmos pressure with the Sartorius membrane filter (type SM 121-36).

Male and female Sprague Dawley rats, aged 2,15 and 26 months, were used. Each experiment was carried out on groups of 10 animals. The aqueous thymus extract was tested in the old rats: the extract was administered by i.p. injection in a total amount of 10 ml for each animal (corresponding to 2 g of fresh tissue), subdivided into 5 doses of 2 ml given on alternate days. To 1 group of control animals an equal volume of saline was administered.

Forty-eight h after the final injection, the animals were sacrificed by bleeding: 16 h before sacrifice the animals were fasted and H^3-uridine (2 Ci/m*M*) was administered at a dosage of 50 μC/100 g of body weight. The determinations of ATP, alkaline phosphatase and cholesterol were effected respectively using the Boeringer Biochemia tests. The concentration of protein levels was determined spectrophotometrically according to LAYNE'S method[8]. DNA and RNA were extracted using the method of MARMUR[9], and KAY et al.[10], modified by SWINDLEHURST et al.[11]. The extraction of DNA was also conducted using the method of SCHNEIDER[12]. The quantitative determinations of DNA and RNA were carried out by the reactions with diphenylamine or orcinol[12]. Aliquots of RNA and DNA samples were used for the determinations of radioactivity by liquid scintillation counting according to BRAY[13].

Results and discussion. ATP, cholesterol, total protein and alkaline phosphatase. From the results reported in Table I, one notes in old untreated rats, as compared to the young ones, a greater amount of cholesterol and total protein and a smaller amount of ATP, which confirms previously published data[14–16]; old rats given aqueous thymus extract show a significant decrease of cholesterol and total proteins and a significant increase of ATP. An examination of the statistical variances demonstrates that the values of ATP cholesterol and proteins of old rats treated with aqueous thymus extract come within the values shown by the young rats. As for the alkaline phosphatase, the aqueous thymus extract causes a considerable and significant decrease of enzymatic activity in old rats as compared to untreated old rats;

Table II. Quantitative determination and specific radioactivity of DNA and RNA extracted according to SWINDLEHURST[11].

Nucleic acid in the liver	Rats	
	26 months (not treated)	26 months (treated)
DNA (mg/g)	1.009 ± 0.097	1.811 ± 0.222
dpm DNA/mg DNA	2710 ± 155	476 ± 42
RNA (mg/g)	9.415 ± 0.447	8.817 ± 1.322
dpm RNA/mg RNA	535 ± 76	397 ± 78

The H^3-uridine was administered at a dosage of 50 μC/100 g of body wt. Mean values ± the average quadratic error of the mean.

[1] L. G. ROWNTREE, J. H. CLARK, A. STEINBERG and A. M. HANSON, J. Am. med. Ass. *103*, 1425 (1934).
[2] F. M. BURNET, *Immunological Surveillance* (Pergamon Press, Oxford 1970).
[3] R. L. WALFORD, *The Immunological Theory of Aging* (Munksgaard, Copenhagen 1969).
[4] P. R. J. BURCH, *Growth, Disease and Ageing* (Oliver and Boyd, Edinburgh 1968).
[5] E. M. PONTELOURIS, Expl. Geront. *7*, 73 (1972).
[6] D. AMICI and G. L. GIANFRANCESCHI, Commun. Congress. Soc. Ital. Biol. Sper., Palermo, 3 October 1971.
[7] G. L. GIANFRANCESCHI and D. AMICI, Commun. Congress. Soc. Ital. Biol. Sper., Palermo, 3 October 1971.
[8] E. LAYNE, Meth. Enzym. *3*, 447 (1957).
[9] J. MARMUR, Meth. Enzym. *6*, 726 (1963).
[10] E. R. M. KAY, N. S. SIMMONS and A. L. DOUNCE, J. Am. chem. Soc. *74*, 1724 (1952).
[11] M. SWINDLEHURST, S. J. BERRY and W. FIRSHEIM, Biochim. biophys. Acta *228*, 313 (1971).
[12] W. C. SCHNEIDER, Meth. Enzym. *3*, 680 (1957).
[13] G. A. BRAY, Analyt. Biochem. *1*, 279 (1960).
[14] K. SALATKA, D. KRESGE, L. HARRIS, D. EDELSTEIN and P. OVE, Expl. Geront. *6*, 25 (1971).
[15] T. F. ZUCKER and M. ZUCKER, J. Nutr. *80*, 20 (1963).
[16] V. V. FROL'KIS, L. N. BOGATSKAYA and E. V. EPSHTEIN, Biokhim. Zh. *38*, 514 (1966).

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.

D. AMICI, G.L. GIANFRANCESCHI, G. MARSILI and L. MICHETTI

Institute of General Physiology, University of Camerino, I-62032 Camerino (Italy), 13 December 1973.

[17] G. B. PRICE, S. P. MODAK and T. MAKINODAN, Science *171*, 917 (1971).

[18] S. R. PELC, Expl. Geront. *5*, 217 (1970).

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