in medium containing or lacking autologous plasma is presented in Table I. From this data 4 main observations can be made and they are: 1. Under all conditions employed, the peak of cortisol uptake occurs within 15 min. 2. A decline follows this peak, this decline is greater at 4 °C, least at 37 °C. 3. At 4 °C the peak uptake is greater than at 37 °C. 4. Total uptake is always greater in systems containing autologous plasma.

The above data suggests that some plasma factor markedly influences the uptake of cortisol. Since our previous studies indicated the importance of plasma in the cortisol mediated inhibition of lymphocyte protein synthesis, and, since it was tentatively suggested that the required plasma factor was transcortin[4,5] (the cortisol binding protein of human plasma[7]), autologous plasma was heated at 60 °C for 15 min in order to destroy the cortisol binding capacity of transcortin[8]. The results presented in Table II indicate that such heating markedly inhibits the uptake of cortisol.

Table I. The uptake of cortisol by cultured CLL lymphocytes and the effect of plasma and temperature

Time (min)	CPM at 4 °C per 10^7 cells		CPM at 37 °C per 10^7 cells	
	+ Plasma S.D.	— Plasma S.D.	+ Plasma S.D.	— Plasma S.D.
5	255 ± 25	125 ± 20	500 ± 40	335 ± 25
15	1820 ± 60	840 ± 65	760 ± 45	400 ± 30
30	400 ± 35	180 ± 30	640 ± 40	275 ± 30

Table II. The effect of heating plasma on the uptake of cortisol by 10^7 CLL cells at 37 °C

Time (min)	CPM Unheated plasma	CPM Heated plasma
15	870	200
30	634	50

Table III. The effect of $10^{-3}M$ DNP on the uptake of cortisol at 4 °C and 37 °C by 10^7 cells

Time (min)	CPM at 4 °C		CPM at 37 °C	
	— DNP	+ DNP	— DNP	+ DNP
15	440	305	385	320
30	280	300	280	280

The results presented in Table I illustrate as previously noted, that the extrusion of cortisol by CLL cells occurs after 15 min of incubation. In order to determine the dependence of this process on cellular energy, CLL cells were first incubated in medium containing DNP ($10^{-3}M$) for 15 min and then H^3-cortisol was added. The results presented in Table III indicate that both the uptake and extrusion of the hormone are inhibited by DNP.

Discussion. The results obtained in this preliminary study suggest that the uptake of cortisol by CLL cells is influenced by some plasma factor (transcortin?), dependent upon cellular energy, and is inversely related to temperature. Although the latter two observations are in agreement with those of MUNCK and BRINCK-JOHNSEN[9], and SCHAUMBURG and BOJESEN[10], the positive influence of a plasma factor on the uptake of the hormone and the extrusion of the hormone are new observations. It is interesting to note that SCHAUMBURG and BOJESEN discuss the remarkable resemblance of the thymic cortisol receptor to transcortin further supporting the possibility that the plasma factor influencing cortisol uptake and perhaps entering the lymphocytes as a complex with the hormone, is transcortin. Further studies are currently in progress whose experimental design has been pointed toward the investigation of the above relationship.

Zusammenfassung. Die Aufnahme von Cortisol in Lymphozyten wird durch ein thermolabiles Plasmaprotein begünstigt.

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Chicken Gizzard, a Myoglobin Containing Smooth Muscle

It has recently been reported that myoglobin is not detectable in human uterine muscle[1,2], and the question has been raised by FASOLD et al.[2] as to whether myoglobin is at all synthesized by smooth muscle cells. We now wish to present evidence that one type of smooth muscle, namely chicken gizzard muscle, appears to contain myoglobin or a myoglobin-like substance.

Chicken gizzard muscle has been established as a smooth muscle by both its morphology[3,4] and its biochemical properties[5,6]. Recently, in our own laboratory, immunochemical studies have shown the antigenic similarity of chicken gizzard myosin to the actomyosins of other smooth muscle fibres and its dissimilarity to the actomyosins of striated and cardiac muscle.

For the preparation of myoglobin extracts, the muscle layer of fresh or frozen chicken gizzards was quickly removed and homogenized at 4 °C with 2 volumes of water. After filtering through gauze, such extracts were centrifuged at 16,000 × *g* for 30 min and the supernatant solutions were either used directly or after lyophilisation and redissolution. When these extracts were applied to a Sephadex G-50 column (2 × 93 cm, equilibrated with 0.01 *M* phosphate buffer, pH 7.4), 2 distinctly separated reddish-brown fractions were eluted, using the same

buffer; the first one appearing with the void volume V_0 of the column (106 ml), the second, a considerably larger one, with an elution volume V_e of 140 ml (Figure 1a). Purified horse hemoglobin and myoglobin (Miles-Seravac),

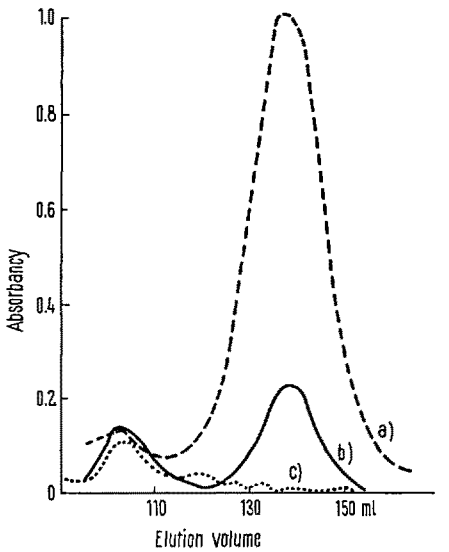

Fig. 1. Sephadex G-50 chromatography of the extracts from a) chicken gizzard (---); b) 'red' leg muscles (——); and c) 'white' pectoral muscle (·····).

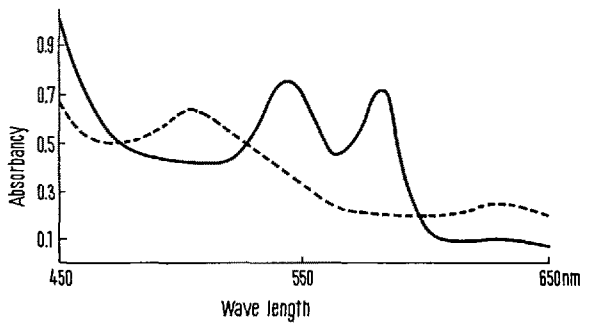

Fig. 2. The spectrophotometric tracing of chicken gizzard 'myoglobin' in the oxy- (——) and met- (---) form.

applied to the same column, were shown to give identical elution patterns. The absorption spectra of the second peak and its met-form (Figure 2) are almost identical with the absorption spectra reported for human myoglobins, e.g.[7]. We therefore conclude from these preliminary experiments that besides hemoglobin, chicken gizzard muscle contains a significantly impressive amount of myoglobin or myoglobin-like substance.

Control experiments, using other chicken tissue, showed that similarly prepared extracts from cardiac or 'red' leg muscle (Mm. adductor magnus and semimembranosus) contained both hemoglobin and myoglobin (Figure 1b); 'white' pectoral muscle hemoglobin and a trace of myoglobin (Figure 1c); whereas myoglobin was virtually absent when extracts from liver or oviduct were applied to the column.

We have recently prepared the myoglobin from gizzard smooth muscle in a quantitative scale for biochemical and immunological studies. These results will be presented shortly.

Zusammenfassung. In der glatten Muskulatur des Hühnermuskelmagens konnte ein wasserlösliches Hämoprotein nachgewiesen werden, das sich chromatographisch und spektrophotometrisch wie Myoglobin verhält.

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Einbau von Schwefelwasserstoff in Verbindungen des Photosynthesestoffwechsels

In photosynthetisierenden Zellen entstehen unter der Einwirkung von Schwefelwasserstoff wasserlösliche Schwefelverbindungen[1]. Eine Identifizierung gelang bisher nicht, da nur geringe Substanzmengen anfallen. Verschiedene Hinweise lassen jedoch vermuten, dass die Verbindungen als Derivate von Zwischenprodukten des zentralen und intermediären Stoffwechsels aufzufassen sind[2,3]. In-vitro-Experimente erlaubten uns, eine Eintrittsstelle in den zentralen Stoffwechsel aufzufinden und die gebildete Verbindung zu identifizieren.

Zu diesem Zweck untersuchten wir die Carboxylierung des Ribulose-di-phosphats (Sigma, Chemical Company, St. Louis, USA) in Anwesenheit von $H^{14}CO_3^-$ und frisch zubereitetem $H^{35}S^-$[4]. Vorversuche haben ergeben, dass die partiell gereinigte Spinatcarboxylase (Sigma) ohne Cofaktoren zur Carboxylierung imstande ist und die verwendete Sulfidkonzentration die Reaktion nicht merklich beeinflusst. Dieser suboptimale Ansatz bezweckt, Artefakte mit dem reaktionsfreudigen Sulfid auf ein Minimum herabzusetzen. In den Hauptversuchen enthielt jeder Ansatz in 1 ml Lösung (pH 7,8) 0,1 Einheit Carboxylase, 2 μM Ribulose-di-phosphat, 10 μM HCO_3^- und 10 μM HS^-, ausgenommen Ansatz No. 1. Die Ansätze 1–3 waren mit ^{14}C und die Ansätze 3–4 mit ^{35}S markiert. Im Anschluss an die 60minütige Inkubation der Reaktionsgemische erfolgte eine papierelektrophoretische Auftrennung der Produkte[5]. Mittels Autoradiographien wurden die einzelnen Verbindungen aufgesucht, anschliessend eluiert, gesammelt und im IR-Spektrophotometer analysiert[6].

Um Artefakte ganz auszuschliessen, wurden alle in Frage kommenden Kombinationen zwischen Edukten,