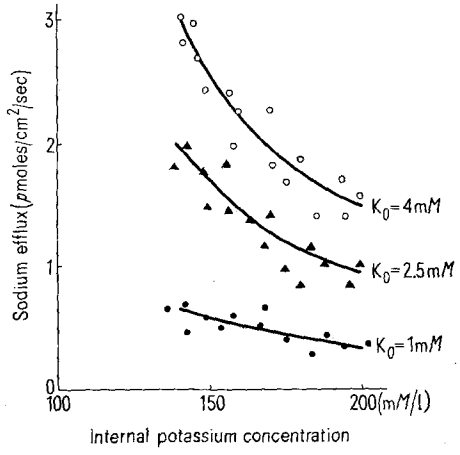
Spectrophotometer with flame attachment. The values for $[K_i]$ have been corrected for K^+-ions in the interspace.

Results. The data given in the Figure show that increasing the concentration of intracellular K^+-ions from 140 m*M* (characteristic for frog sartorius in physiological Ringer[3]) to up to 200 m*M* markedly inhibits active sodium extrusion. Independent of the external potassium level, the Na^+-efflux is reduced to about half the original rate. The reciprocal plot of the Na^+-efflux rate against $[K_0]$ at different internal potassium levels indicates that the internal potassium acts competitively. At the $[K_i]$ of 140 m*M*, the maximal rate of Na^+-efflux obtained from the LINEWEAVER-BURK diagram is 25 pmoles/cm² per sec.



Inhibition of active sodium efflux by internal potassium at different levels of external potassium ions. Paired muscles have been immersed in Ringer's solution[3,8], the potassium concentration being varied between 1–4 m*M* as indicated. The immersion solution for one of the twin muscles contained 10^{-5}*M* strophantidin; 20 min equilibration time was followed by a 45 min period, during which the sodium efflux rates with and without strophantidin were measured. The values given in the Figure represent the differences of the average values plotted against the average internal potassium levels. The solid curves are the least-square fit to the individual experiments indicated by the respective symbols.

Discussion. At the normal internal potassium level of 140 m*M*, where a direct comparison with the work of SJODIN[6] becomes possible, the observed kinetics of active sodium extrusion are rather similar. Increasing the intracellular potassium level results in a marked inhibition of the Na^+-efflux. As a possible mechanism, it is suggested that the intracellular K^+-ions may induce a conformation of the Na-K-ATPase which has only a low affinity for ATP[9]. On the basis of the evaluated Hill coefficient, it seems likely that 2 K^+-ions participate in this inhibitory reaction.

In view of the controversy existing in the literature as regards the intrafibrillar activities of Na^+ and K^+ in frog muscle[10] (though high concentrations close to the internal membrane surface would suffice), no attempt has been made to fit the data to a quantitative kinetic model. The possible regulatory role of the internal potassium may be to prevent complete Na^+-extrusion, when the $[K_i]/[Na_i]$ ratio is very high. Such a mechanism would have its counterpart in the inhibitory action observed for high external sodium concentrations[6].

Zusammenfassung. Radioaktive Untersuchungen zeigen, dass intrazelluläre K^+-Ionen den aktiven Na^+-Austausch hemmen.

R. A. CHAPLAIN[11]

Manfred von Ardenne Research Institut. Zeppelinstr. 7, DDR–8051 Dresden-Weisser Hirsch (DDR), 27 December 1973.

[9] C. HEGYVARY and R. L. POST, J. biol. Chem. *246*, 5234 (1971).
[10] W. McD. ARMSTRONG and C. O. LEE, Science, N.Y. *171*, 413 (1971).
[11] Present address: Technische Hochschule Otto von Guericke, Abteilung für Biokybernetik, Bierut-Platz 5, DDR-301 Magdeburg.

Dexamethasone Effects on Liver β-Glucuronidase and Tryptophan Oxygenase Activities in Rats of Different Ages

The factors that regulate the lysosomal enzyme activity in tissues are poorly understood. The β-glucuronidase, a representative lysosomal enzyme, is under androgen control in the kidney[1], but in other tissues production of this enzyme appears constitutive[2]. The possibility exists that these enzymes may be under glucocorticoid regulation. These hormones decrease the activity of various hydrolases in the liver and intestine during growth[3,4] and in lymphocytes and monocytes during their in-vitro transformation[5,6]. The naturally occurring and synthetic glucocorticoids like dexamethasone have a stabilising effect on lysosomal membranes[7,8] and their administration to cells in vitro produces a striking prolongation of life-span[9].

The effects of dexamethasone administration in vivo on β-glucuronidase activity and distribution in the lysosomal, microsomal and supernatant (cytosol) fractions of the liver of intact young and senescent rats and, for comparison, on a glucocorticoid-inducible liver enzyme such as the tryptophan oxygenase[10,11] have been examned.

Male Wistar rats, 4 and 24 months old, fed ad lib. until death, were employed and divided in 2 groups. One group was treated daily with i.p. injections of dexamethasone-21-phosphate (Decadron, Merck, Sharp and Dohme) for 72 h and 4 mg/kg total dose, while the control group was injected with the solvent. 96 h after the injections started, the animals were killed by cervical dislocation and the livers were excised, divided in 2 parts and weighed.

One part of the liver was employed for β-glucuronidase (EC 3. 2. 1. 31) determination by the following procedure.

[1] K. KATO, H. IDE, T. SHIRAHAMA and W. H. FISHMAN, Biochem. J. *117*, 161 (1970).
[2] R. DOFUKU, U. TETTENBORN and S. OHNO, Nature new Biol. *232*, 5 (1971).
[3] K. M. WANG, M. BALZER, K. BERDE and M. FOLDVARY, Fedn Proc. *29*, 348 (1970).
[4] O. KOLDOVSKY and M. PALMIERI, Biochem. J. *125*, 697 (1971).
[5] A. C. ALLISON, Nature new Biol. *230*, 163 (1971).
[6] E. WIENER and Y. MARMARY, Lab. Invest. *21*, 505 (1969).
[7] G. WEISSMANN, New Engl. J. Med. *20*, 1143 (1965).
[8] A. M. SYMONS, D. A. LEWIS and R. J. ANCILL, Biochem. Pharmac. *18*, 2581 (1969).
[9] G. C. YUAN and R. S. CHANG, J. Geront. *24*, 82 (1969).
[10] R. T. SCHIMKE, E. W. SWEENEY and C. M. BERLIN, J. biol. Chem. *240*, 322 (1965).
[11] T. BERG, D. BOMAN and P. O. SEGLEN, Expl Cell Res. *72*, 571 (1972).

Table I. β-glucuronidase activity (means ± S.E.) in the liver fractions of young and old control and dexamethasone treated rats

Fractions	4-month-old rats (4) Control	Dexamethasone	24-month-old rats (4) Control	Dexamethasone
Lysosomal	48.7 ± 1.72	49.7 ± 0.81	47.7 ± 1.40	48.9 ± 1.33
Microsomal	49.1 ± 2.26	45.1 ± 0.38	43.5 ± 2.49	42.5 ± 0.49
Supernatant	17.8 ± 1.79	11.2 ± 0.50	10.8 ± 0.23	8.6 ± 1.21
Total activity	115.6 ± 5.77	106.0 ± 1.69	102.0 ± 4.12	100.0 ± 3.03

Data are expressed as mg phenolphthalein liberated/h per g fresh wt. Numbers in parentheses are numbers of determinations.

Table II. Tryptophan oxygenase activity (means ± S.E.) in the liver of young and old control and dexamethasone treated rats

Incubation (min)	4-month-old rats (7) Control	Dexamethasone	24-month-old rats (7) Control	Dexamethasone
0	0.89 ± 0.244	0.99 ± 0.179	0.86 ± 0.216	1.46 ± 0.173
10	0.87 ± 0.224	1.08 ± 0.167	0.86 ± 0.191	1.50 ± 0.156
20	1.01 ± 0.218	1.12 ± 0.142	0.93 ± 0.195	1.60 ± 0.123
40	1.21 ± 0.181	1.46 ± 0.178	1.20 ± 0.168	1.87 ± 0.095
60	1.54 ± 0.188	1.85 ± 0.208	1.46 ± 0.122	2.12 ± 0.121

Data are expressed as mmoles kynurenine produced per g fresh wt. at the times indicated. Numbers in parentheses are numbers of determinations.

The liver portion was homogenized as a 15% (w/v) suspension in 0.25 *M* sucrose-0.05 *M* phosphate buffer (pH 7.4)-0.001 *M* EDTA, in a loose-fitting Teflon pestle glass homogenizer at 0–4°C. After centrifugation at 750 *g* for 10 min to eliminate nuclei and cell debris, the supernatant was centrifuged at 12,000 *g* for 20 min to obtain the lysosomal fraction. Microsomes were then obtained by centrifuging the postlysosomal supernatant at 105,000 *g* for 60 min in a Spinco preparative ultracentrifuge. After treatment of the lysosomal and microsomal pellets with Triton X-100 0.2% final concentration, the β-glucuronidase activity was determined on both these fractions and the 105,000 *g* supernatant as described previously[12].

For tryptophan oxygenase (EC 1.13.1.12) determination, the second portion of the liver was homogenized as a 1% (w/v) suspension in 0.02 *M* phosphate buffer (pH 7.0), containing 2.5 m*M* L-Tryptophan and 2 μ*M* denatured hemoglobin (Serva, Heidelberg), at 0–4°C. After centrifugation at 750 *g* for 10 min, the supernatant was employed for tryptophan oxygenase determination according to SEGLEN and JERVELL[13].

Table I shows that dexamethasone reduces the β-glucuronidase release by liver lysosomes mainly in young rats ($P < 0.05$ vs. young controls) and decreases also the microsomal activity of this enzyme in these animals ($P < 0.05$ vs. young controls). In young rats the total enzyme activity also decreases with dexamethasone. Old animals show, on the contrary, minor changes, statistically not significant. The microsomal activity of β-glucuronidase appears therefore to be modified by steroid treatment only in young rats, as previously demonstrated also for acid phosphatase in the chick[3].

Table II shows that the tryptophan oxygenase induction by dexamethasone in rat liver is higher and statistically significant in senescent animals ($P < 0.01$ vs. old controls and $P < 0.05$ vs. young treated rats). The old rat appears therefore capable of responding to hormone administration with an increase in liver specific protein biosynthesis greater than that of the younger one. This is in keeping with previous observations[14] and confirms earlier reports which are against the presence of a generalized impairment of gene function and/or information flow in the liver of ageing rats[15]. In view of the possibility that histones may act as regulators of gene activity[16], the finding that steroid hormones bind to these nuclear structures[17] appears important for explaining the present observations.

The results here reported suggest that a glucocorticoid treatment may decrease the synthesis of lysosomal enzymes[18] in the liver cells of young rats only. Old animals appear not to respond in a similar way. Since these hormones stimulate the synthesis of certain of the liver enzymes, as also shown here, the reduced formation of beta-glucuronidase in young rats appears more probably due to a reduced release of the hydrolase from lysosomes, that is to a feedback mechanism brought about by the hormone, rather than to a primary inhibitory effect at the microsomal level.

Riassunto. La sintesi di β-glucuronidasi nel fegato di ratti di 4 e 24 mesi di vita trattati con desametasone-21-fosfato viene apparentemente diminuita negli animali giovani e non in quelli senescenti. In questi ultimi si osserva al contrario una stimolazione della sintesi di triptofano ossigenasi superiore a quella presentata dagli animali di giovane età.

R. COMOLLI and M. E. FERIOLI

Istituto di Patologia generale dell'Università di Milano, e Centro di Patologia cellulare del C.N.R. Via Mangiagalli 31, I-20133 Milano (Italy),
12 December 1972.

[12] R. COMOLLI, M. E. FERIOLI and S. AZZOLA, Expl Geront. 7, 369 (1972).
[13] P. O. SEGLEN and K. F. JERVELL, Biochim. biophys. acta *171*, 47 (1969).
[14] R. C. ADELMAN, Expl Geront. *6*, 75 (1971).
[15] C. E. FINCH, Expl Geront. *7*, 53 (1972).
[16] V. G. ALLFREY, in *Histones and Nucleohistones* (Ed. D. M. P. PHILLIPS; Plenum Press, London and New York 1971), p. 241.
[17] M. SLUYSER, Biochem. J. *130*, 49 P (1972).
[18] J. L. VAN LANCKER and P. L. LENTZ, J. Histochem. Cytochem. *18*, 529 (1970).