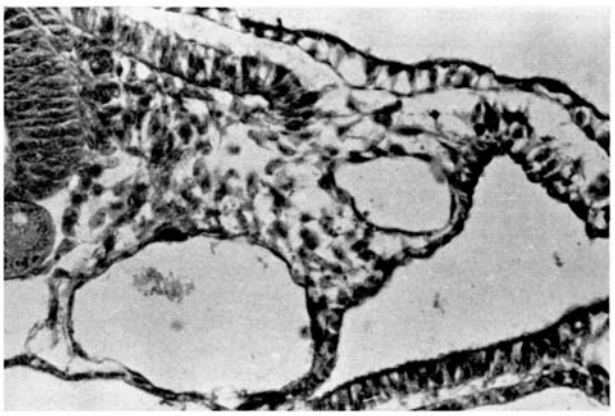

Fig. 1

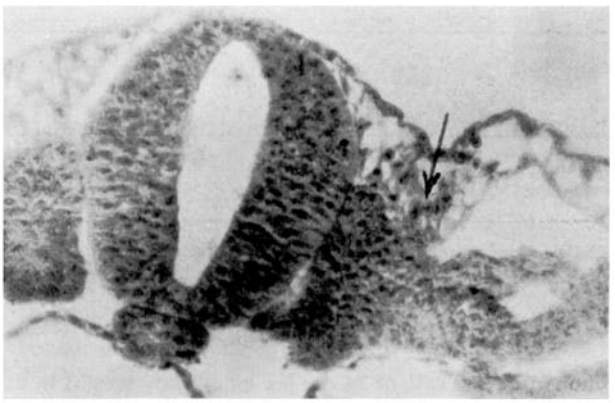

Fig. 2

nephros has been confirmed by various authors[2,6,7]. Besides this, the failure of the ontogeny of mesonephros has another aspect to be considered. Mesonephros can be formed out of the competent mesodermal cells through the inducing process from the neural area and the notochord[14] which become normally developed in almost all the embryos studied in this experiment. Thus the failure of the formation of the mesonephros may be due to two reasons – one the absence of the inducing substance generally conveyed through the pronephric duct, and the other the absence of competence of the intermediate mesoderm, though the cells in this area may get the normal evocation from the chorda and the neural area.

Though, in the majority of the cases, neither the pronephros nor the pronephric duct develop, in some of the experimented embryos a solid duct is formed independently in the absence of tubules. This is in accordance with the findings of KUME[4] and CAMBER[5]. The pronephric tubules and the duct develop independently by self-differentiation and the tubules become secondarily attached to the duct.

Zusammenfassung. Chloramphenicol, auf verschiedene Ontogenesestadien des Hühnchens angewendet, hemmt bei den meisten Embryonen die Nierenentwicklung selektiv, während bei einigen ein pronephritischer Gang unabhängig von den Tubuli differenziert wird.

A. BOSE

Department of Zoology of the University of Kalyani (Nadia, W. Bengal, India), November 24, 1964.

[14] C. H. WADDINGTON, *Principles of Embryology* (Cambridge University Press, 1956), p. 268.

Physiological Activities of Chondroitin Polysulfate: the Short-Term Effect of Intravenous Injection

Long term administration of synthetic chondroitin polysulfate (ChPS) has reduced serum total lipid and cholesterol and retarded the atherosclerotic process in experimental animals[1]. It was postulated that the lipid clearing activity of ChPS was correlated with the sulfur content of this substance[1]. Anticoagulant activity of ChPS has also been associated with sulfur content on the basis of infrared spectroscopic evidence[2].

Physiological changes in the serum components were also demonstrated in hyperlipemic rabbit serum a short time after the intravenous administration of ChPS. The short term effects of ChPS were determined in the following manner.

ChPS was prepared synthetically by sulfation of chondroitin sulfate (ChS) from shark cartilage[1,2]. Analysis of the ChPS lot used principally in this investigation showed 16.09% sulfur, 2.16% nitrogen and $[\alpha]_D^{20} - 15.0°$ (c 1.0 H_2O). 17 male albino rabbits averaging 2.9 kg were fed 0.1 g cholesterol per kg of body weight daily in a basic diet. After 6 weeks of cholesterol feeding, an elevation of the serum total cholesterol from approximately 40 mg initially to over 300 mg per 100 ml was attained. At this time single injections of ChPS were administered intravenously into the hyperlipemia-induced rabbits at the rate of 2 to 10 mg per kg of body weight. The blood was drawn at desired intervals to test the serum for turbidity, total and ester cholesterol, and blood coagulation time as reported previously[1,3]. Horizontal paper electrophoresis was carried out on Whatman no. 1 paper wetted with Veronal buffer, pH 8.6. 25 μl of serum was applied and a voltage of 350 V (current density 0.4 mA/cm) was used for 5 h. After drying the paper at 90°C, lipoproteins were located by staining the strip with Sudan Black (Merck) in 50% ethanol for $2^1/_2$ h.

Effects of ChPS on turbidity and total cholesterol in hyperlipemic serum during the short term investigation are shown in the Table. When ChPS was injected intra-

[1] K. MURATA, Naturwissenschaften *49*, 39 (1962).
[2] K. MURATA, Nature, Lond. *193*, 578 (1962).
[3] K. MURATA, J. Gerontol. *17*, 30 (1962).

Effects of chondroitin polysulfate on turbidity and total cholesterol in hyperlipemic serum

	Initial value[a]		Value[a] after injection			
			30 min		60 min	
Turbidity[b]	0.130 ± 0.008	100%	0.104[c] ± 0.008	80.0%	0.103[c] ± 0.011	79.2%
Total cholesterol (mg%)	341 ± 32	100%	290 ± 23	85.0%	311 ± 17	91.2%

[a] These values represent the mean and standard error, and percentage of initial value. [b] Turbidity expressed as optical density [4].
[c] Difference between the mean and that of the initial value is significant ($p < 0.05$).

venously at the rate of 10 mg per kg of body weight in 11 rabbits, serum turbidity was reduced approximately 20% and the difference between the initial turbidity and the turbidities at 30 min and 60 min was significant at the 0.05 level. However, no significant changes were found in serum turbidities determined more than 60 min after ChPS injection. The injection of ChPS similarly reduced the serum total cholesterol, but not significantly. The ratio of serum ester to total cholesterol did not change markedly in this period. None of these changes in the serum occurred in hyperlipemia-induced control rabbits injected with physiological saline.

Paper electrophoresis indicated that a single injection of ChPS into hyperlipemic rabbits resulted in an acceleration of serum lipoprotein mobilities during the first hour after injection, but did not noticeably alter the β/α lipoprotein ratios. However, the degree of acceleration of the lipoprotein migration varied to some extent on different occasions within the same animal or between animals. There was no increase of lipoprotein mobility in rabbits with total cholesterol levels less than 100 mg per 100 ml. Figure 1 presents a typical electrophoretic pattern following the injection of 10 mg ChPS per kg of body weight into hyperlipemic rabbit (354 mg per 100 ml serum total cholesterol); migration of both the α- and β-lipoprotein bands was accelerated at 30 min and 60 min, and then normalized gradually.

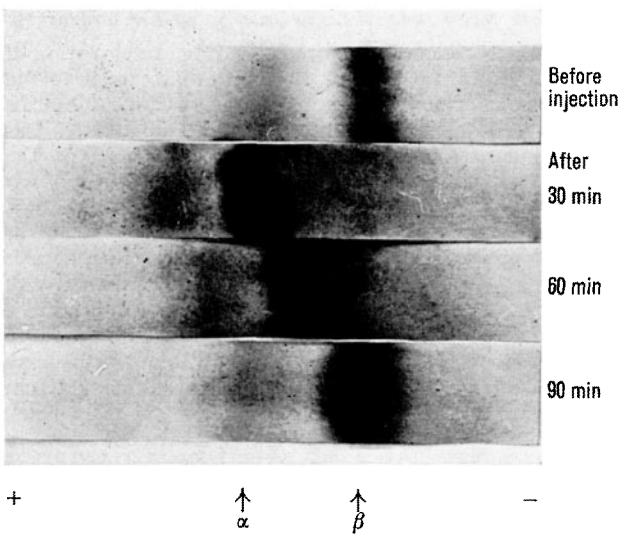

Fig. 1. The short term effect of chondroitin polysulfate on α- and β-lipoprotein mobility as recorded by paper electrophoresis. Accelerated migration of serum lipoproteins is shown at 30 min and 60 min after intravenous injection. Stained with Sudan Black.

The single intravenous injection of ChPS produced a definite anticoagulant activity during the first hour, which corresponded to the amount of ChPS administered (Figure 2). The maximum activity was shown at 15 and 30 min and diminished gradually after 60 min. A single injection of 5 mg ChPS per kg of body weight prolonged the clotting time to 180 min in blood samples taken at 15 and 30 min. When animals were given a dose of 10 mg ChPS, the samples taken at 15, 30 and 45 min did not clot within the 200 min test period.

Thus reduction of serum turbidity and cholesterol and acceleration of lipoprotein mobility resulting from the administration of ChPS are apparently correlated with the coincidental anticoagulant activity. No serum lipid changes resulted from the injection of ChPS into normal animals not treated by cholesterol feeding.

It appears that a single injection of ChPS produces a physiological activity which disappears at a fairly rapid rate in vivo. It is known that the anticoagulant activity of heparin disappears within several hours after intravenous injection of approximately 1 mg per kg of body

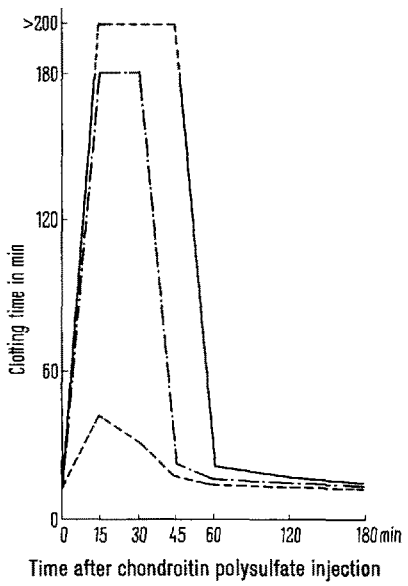

Fig. 2. Anticoagulant activity of chondroitin polysulfate containing 16.09% sulfur. Clotting time was measured by LEE and WHITE method[4]. Doses of ChPS are —— 10 mg/kg of body weight, ---- 5 mg/kg, ----- 2 mg/kg.

[4] R. BIGGS and R. G. MACFARLANE, *Human Blood Coagulation and its Disorders*, III Ed. (F. A. Davis Co., Philadelphia 1962), p. 380.

weight. 20% of $S^{35}O_4$-labeled heparin has been reported to appear in dog urine 2 h after injection[5]. Our previous experiment[6] showed that 84% of $S^{35}O_4$-labeled ChS biosynthetically obtained from rat cartilage was excreted in rabbit urine 7 h after intravenous injection. KAPLAN and MEYER[7] have recently reported that ChS-A and C disappeared from the blood of man and dog in less than 4 h when a dose of approximately 1 mg/kg of body weight was injected intravenously; 80% of ChS disappeared 2 h after injection. It appears that ChS-A and C as well as heparin remain in circulating blood several hours when administered intravenously. The degradation rate in vivo of ChPS is apparently similar to ChS-A or C and probably to heparin.

The mechanism of the physiological activity of ChPS is not clear. Acceleration of the electrophoretic mobility of the serum lipoproteins has been reported following the administration of heparin[8], dextran sulfate[9] and other sulfated polysaccharides[10]. This effect may be associated with lipid clearing activity. Since it is known that certain sulfated polysaccharides administered in vivo produce a lipid clearing activity[11–13], the serum alterations resulting from ChPS injection may be correlated with the production of a clearing factor[14].

Zusammenfassung. Chondroitin-Polysulfat führt nach intravenöser Injektion bei hyperlipämischen Kaninchen kurzfristig zu Verminderung der Turbidität und des Cholesteringehaltes im Serum und Beschleunigung der elektrophoretischen Mobilität des Serumlipoproteins.

K. MURATA[15]

Department of Physical Therapy and Medicine, Faculty of Medicine, University of Tokyo (Japan),
December 6, 1964.

[5] T. A. LOOMIS, Proc. Soc. exp. Biol. Med. *106*, 490 (1961).
[6] F. HARUKI, K. MURATA, and H. DEBUCHI, J. Jap. Rheumat. Assoc. *2*, 453 (1960).
[7] D. KAPLAN and K. MEYER, J. clin. Invest. *41*, 743 (1962).
[8] S. GERÖ, M. BAUMAN, M. JAKAB, S. ROHNY, and J. GÁCS, Z. ges. exp. Med. *129*, 335 (1957).
[9] S. M. JEAVONS and C. R. RICKETTS, J. clin. Pathol. *9*, 255 (1956).
[10] P. BERNFELD, J. S. NISSELBAUM, B. J. BERKELEY, and R. W. HANSON, J. biol. Chem. *235*, 2852 (1960).
[11] E. D. KORN, in *Methods of Biochemical Analysis* (Interscience Publishers Inc., 1959), vol. 7, p. 152.
[12] D. S. ROBINSON, P. M. HARRIS, and C. R. RICKETTS, Biochem. J. *71*, 286 (1959).
[13] L. LEVY and G. E. CRONHEIM, Biochem. Pharmacol. *7*, 271 (1961).
[14] Acknowledgment: I am greatly indebted to Prof. Y. OSHIMA for his interest and advice in this work, and to Dr. T. FURUHASHI for providing chondroitin polysulfate.
[15] Present address: Department of Medicine, Loma Linda University School of Medicine Los Angeles (California, USA).

On the Interaction of Glucocorticoids and Anabolic Steroids

Anabolic steroids inhibit the proteino-catabolic action of glucocorticoids[1] and prevent the atrophy of adrenal cortex induced by these hormones[2]. The effects of anabolics and glucocorticoids on the fat and glycid metabolism have been studied up to now only after separate administration[3–6]. The lipaemic level in serum and glycogen content in liver are significantly increased by glucocorticoids[3–5]. Following the treatment with anabolic-androgenic steroids, these levels either remain unchanged or they decrease[5,6]. Apparently the type of drug, dosage, and time of action of the steroid play an important role.

We therefore decided to contribute to the elucidation of the problem of interaction of both types of hormones. In our experiment we used 19-nortestosterone phenylpropionate (NTPP) as the anabolic steroid and hydrocortisone acetate as the glucocorticoid.

The four groups of 10 male rats of Wistar-Konárovice strain (weiging 180–220 g) received daily subcutaneous doses of (A) 0.4 ml olive oil, (B) 2 mg NTPP, (C) 2 mg hydrocortisone, and (D) 2 mg both of NTPP and hydrocortisone, all per 100 g body weight, for 10 consecutive days. The compounds were dissolved in oil, the volume of which was equal for all the groups including the controls (A). 48 h after the last injection, and after 24 h of starvation, the animals were decapitated; the total lipaemia in the serum[7] and the soluble and insoluble glycogen fractions in the liver[8,9] were determined. The statistical evaluation was accomplished by the analysis of variance and by the test of mean value concordance[10]. The confidential limits of the means are always mentioned.

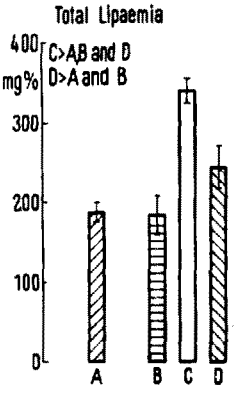

Fig. 1. The level of total lipaemia in serum after ten days' treatment with NTPP and hydrocortisone. A, control; B, NTPP; C, hydrocortisone; D, NTPP + hydrocortisone. The differences are significant at $p = 0.05$.

[1] A. L. BEYLER and G. O. POTTS, First International Congress of Endocrinology Copenhagen, Abstracts (1960), p. 829.
[2] U. K. RINNE and E. K. NÄÄTÄNEN, Acta endocrin., Kbh. *27*, 423 (1958).
[3] A. DURY, Ann. N.Y. Acad. Sci. *72*, 870 (1959).
[4] W. C. HESS and I. P. SHAFFRAN, Proc. Soc. exp. Biol. Med. *83*, 804 (1953).
[5] J. A. THOMAS, Metabolism *13*, 63 (1964).
[6] R. P. HOWARD and R. H. FURMAN, J. clin. Endocrin. *22*, 43 (1962).
[7] J. H. BRAGDON, J. biol. Chem. *190*, 513 (1956).
[8] O. WALLAAS and E. WALLAAS, J. biol. Chem. *187*, 769 (1950).
[9] M. SOMOGYI, J. biol. Chem. *195*, 19 (1952).
[10] E. WEBER, *Grundriss der biologischen Statistik* (VEB G. Fischer Verlag, Jena 1961).