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.

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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.

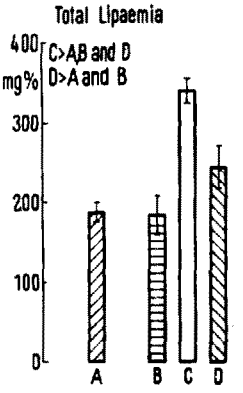

Fig. 1. The level of total lipaemia in serum after ten days' treatment with NTPP and hydrocortisone. A, control; B, NTPP; C, hydrocortisone; D, NTPP + hydrocortisone. The differences are significant at $p = 0.05$.

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NTPP did not affect the lipaemic level (B); hydrocortisone caused a pronounced hyperlipaemia (C) which was decreased by the simultaneous administration of NTPP (D). Figure 2 reveals that hydrocortisone caused an elevation of both glycogen fractions in the liver (C), but this increase was partially inhibited by the simultaneous administration of NTPP (D). NTPP alone did not affect the glycogen level (B).

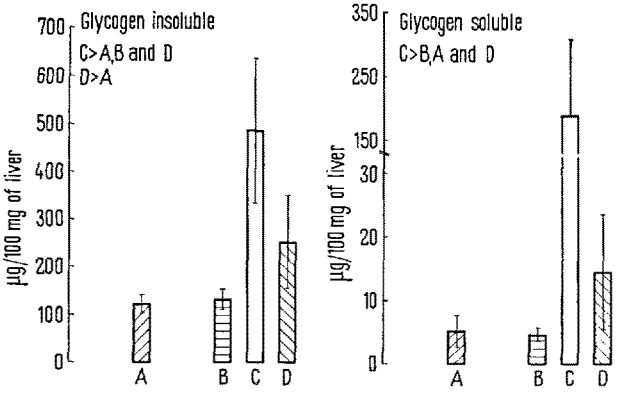

Fig. 2. The level of soluble and insoluble glycogen in liver after 10 days' treatment with NTPP and hydrocortisone. A, control; B, NTPP, C, hydrocortisone; D, NTPP + hydrocortisone. The differences are significant at $p = 0.05$.

Since the increase of both the lipid level in blood and the glycogen content in liver is a concomitant phenomenon of the proteino-catabolic action of glucocorticoids, the decrease both of lipaemia and of glycogen content caused by NTPP could be taken for an anticatabolic effect. This fact can serve as an explanation of why NTPP alone does not exert a substantial influence on lipaemia and glycogen levels in liver.

Zusammenfassung. Der durch Hydrokortison (zehntätige Zufuhr) erhöhte Spiegel der gesamten Lipämie im Serum und der löslichen und unlöslichen Glykogenfraktion in der Leber der Ratte wurde mittels gleichzeitiger 19-Nortestosteron-phenylpropionatzugabe erniedrigt, während 19-Nortestosteron-phenylpropionat allein die verfolgten Parameter nicht beeinflusste.

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Research Institute for Natural Drugs, Prague (Czechoslovakia), January 25, 1965.

Demethylation in vivo of Natulan, a Tumour-Inhibiting Methylhydrazine Derivative

The metabolism of the tumour-inhibiting methylhydrazine derivative RO 4-6467[1] (trade name: Natulan) has been investigated by OLIVERIO and KELLY[2] and by RAAFLAUB and SCHWARTZ[3]. These authors have found a very rapid dehydrogenation of the drug in the plasma and the appearance in the urine of N-isopropylterephthalamic acid. Furthermore it may be of interest that, in vitro, autoxidation of benzylhydrazine leads to the formation of monomethylhydrazine (BERNEISet al.[4]). N-demethylation of Natulan has been postulated by WEITZEL et al.[5,6]. These authors were able to detect formaldehyde after oxidation of the drug in vitro. The metabolism of iproniazid[7] is an example of dealkylation of an alkylhydrazine derivative.

Removal of the 1-methyl group and formation of N-isopropylterephthalamic acid suggests that the Natulan molecule is ultimately cleaved at both sides of the hydrazine group. However, the possibility of the splitting of the N–N bond, according to the classical example of prontosil[8], should also be considered.

This paper reports on the in vivo demethylation of 1-$^{14}CH_3$-Natulan in normal mice and rats, and in rats after pretreatment with phenobarbital. In addition, results obtained by perfusion of isolated rat livers are reported.

Methods. Male Swiss Albino mice weighing 20 g and male rats of 140–200 g (strain 'Füllinsdorf', Hoffmann-La Roche & Co.) were used for the in vivo experiments. Rats from the same source but weighing 250–280 g were used in the perfusion experiments.

All in vivo experiments were initiated by intraperitoneal administration of a single dose of 1-$^{14}CH_3$-Natulan. The metabolic cage and CO_2-trapping devices have been described recently[9]. The perfusion apparatus was a further development of the one described by MILLER et al.[10]. Radioassay was conducted in a TRICARB-liquid-scintillation spectrometer (Packard, Mod. 314 EX).

Results (see Tables). The total expired $^{14}CO_2$ was 19–21% after 24 h in mice and 19% after 12 h (extrapolated: 21% after 24 h) in rats in relation to the original radioactivity administered.

Three perfusion experiments with a perfusate containing 1-$^{14}CH_3$-Natulan (1 mM/l) yielded the following rates of $^{14}CO_2$-production at the steady state: 1.20, 1.25, and 1.43 μM/h/100 g body weight (mean value: 1.29). This corresponds to 75–80% of the mean $^{14}CO_2$ expired following administration in vivo (see Tables 2 and 3). The $^{14}CO_2$ values obtained by the perfusion technique decreased to 2% of the liver perfusion values when the circulating perfusate by-passed the liver.

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