The inhibition of DNA synthesis by hydrocortisone is also observed when ^{14}C-orotic acid is used as the labelled substrate. The data in the Table show that the percent inhibition of the incorporation of label from ^{14}C-orotic acid, which must enter DNA via the de novo pathway, is essentially identical to that observed when ^{3}H-thymidine, which enters via the salvage pathway, is used. In these experiments a single dose of hydrocortisone (50 mg/kg) was administered 19 h post-operative. Either 10 μc of ^{3}H-thymidine or 5 μc of ^{14}C-orotic acid was administered 26 h after partial hepatectomy and 1 h before removal of the liver. Separate experiments confirmed that the incorporation of label from ^{14}C-orotic acid into DNA was linear for 1–2 h, and that the incorporation of label into the acid-soluble fraction 0.5 h after the administration of either precursor, was 5–10% higher in the hydrocortisone-treated rats as compared to the controls. The values for both the control and treated rats have been corrected for the presence of a small amount of RNA contamination in the DNA preparations, which contains label from the ^{14}C-orotic acid. The small error (about 12%) was estimated by extracting the DNA from the 12 h regenerating liver of rats treated as above; label from ^{14}C-orotic acid would appear only in the RNA at this time since DNA synthesis is negligible. The conclusion that the changes in the labelling pattern of DNA are a true indication of the degree of inhibition of DNA synthesis was also supported by the results of mitotic counts. Mitoses were absent in eosin-stained 10 μ sections from the 30 h regenerating liver of rats which received a single dose of 50 mg/kg or 100 mg/kg of hydrocortisone 19 h after partial hepatectomy; approximately 3.5% of the cells were in metaphase in similar preparations from control rats.

These results indicate that the regenerating liver is most sensitive to the steroid if the latter is administered during the period of rapid DNA synthesis in the parenchymal cells, which occurs approximately 17–19 h post-operation in young adult rats[18]. It is this latter finding which may be pertinent to the problem of optimizing schedules for the use of corticosteroids as cancer chemotherapeutic agents, since DNA synthesis shows a diurnal variation in some tumors[22]. Whether some tumors grown in a host animal exhibit this marked sensitivity to corticosteroids when they are in the vicinity of the S-phase must await further study[23].

Percent inhibition of DNA synthesis

Precursor	Specific activity ± S.E.[a] Control	Treated	% inhibition
^{3}H-thymidine (10 μc)	50 ± 3.7 (18)	11 ± 0.89 (9)	78
^{14}C-orotic acid (5 μc)	13 ± 1.9 (7)	3 ± 0.75 (7)	77

[a] Number of rats shown in parenthesis.

Résumé. Dans le foie du rat en voie de régénération, la biosynthèse de DNA est inhibée au maximum par le hydrocortisone 17–19 h après l'opération. C'est pourquoi le foie en régénération est le plus sensible au hydrocortisone s'il est injecté pendant la période de la synthèse maximale de DNA.

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Fatty Acid Unsaturation and Cholesterol Content in Normal and Denervated Muscle

In the denervation atrophy of muscle, a marked increase of the extent of phospholipid labelling from P^{32}-ortophosphate occurs, together with an increased incorporation of C^{14}-acetate into lipids[1–3].

To evaluate differences in lipid composition between normal and denervated muscles, experiments have been carried out on the degree of unsaturation of fatty acids and the ratio cholesterol–phospholipid, which appear to be both relevant to the functional properties of membranes[4–6].

Wistar rats (200–250 g) were used in all experiments. They were maintained on stock diet and allowed free access to food. To produce atrophic muscles, the rats were anaesthetized with ether and the left sciatic nerve of each was cut. In others, the left gastrocnemius muscle was tenotomized. The animals were used 8–18 days after the operation.

Three to five atrophic or control muscles were pooled and homogenized in chloroform-methanol (2:1), the lipids were extracted (under nitrogen) according to FOLCH et al.[7] and redissolved in chloroform for the iodine number determination following the method of WIJS[8]. In parallel experiments the total cholesterol was determined (8 days after the operation) according to the procedure of BLOOR[9].

The results presented in Table I show that in the denervation atrophy the degree of fatty acid unsaturation is significantly lowered, whereas no change has been found in tenotomized muscles.

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Table I

No. of animals	Days after operation	Weight of muscles (g)		Lipid content (mg/g of wet wt.)		Iodine number (mg/100 g of lipid)	
		Control	Denervated	Control	Denervated	Control	Denervated
5	8	6.722	4.444	14.3	16.2	180	131
4	18	5.335	3.025	17.2	20.0	202	143
4	10	4.430	2.915	14.7	17.5	244	186
4	13	4.917	3.160	13.2	16.7	195	167
		Control	Tenotomized	Control	Tenotomized	Control	Tenotomized
3	8	3.625	2.990	17.1	15.3	216	210

The total cholesterol is significantly increased after denervation but not after tenotomy. The mean value for normal muscle (expressed as μg/g of wet wt.) was 1.119 ± 0.095 S.E.M. and for denervated muscle 1.420 ± 0.132 from 6 experiments (18 animals). A strong increase in cholesterol content was found when the microsomal fractions instead of whole denervated muscles were used (Table II). No significant change in phospholipid content per mg of microsomal protein has been found in denervated muscle.

The results point to the existence of differences in the lipid constituents of membranes of normal and denervated muscles. Preliminary observations by means of thin-layer chromatography reveal[10] that the difference in fatty acid saturation involves the phospholipids of whole muscles and of their subcellular fractions. Variations in the chemical structure of the paraffinic chains of the phospholipids have significant consequences in the physicochemical state of the lipids in membranes. The unsaturated bonds appear to prevent a highly condensed state of lipid molecules[4,5].

It is noteworthy that the so-termed condensing effect of cholesterol in mixed-films depends on the nature of hydrocarbon tails of the phospholipid in the system. For instance, cholesterol present in a monolayer with a phospholipid containing *cis*-unsaturated chains produces a condensing effect on the monolayer[11].

Research is now in progress to elucidate some qualitative change in fatty acid composition of normal and denervated muscles and in the dynamic processes of phospholipids[12].

Table II

No. of animals	Weight of muscles (g)		Cholesterol μg/mg of microsomal protein[a]	
	Control	Denervated	Control	Denervated
3	3.290	2.320	14.67	35.70
3	3.700	2.460	14.41	42.00
3	3.824	2.668	15.45	37.90
3	3.403	2.181	16.00	40.50
3	4.010	2.080	11.80	19.55
	Control	Tenotomized	Control	Tenotomized
3	3.431	2.990	15.40	17.00

[a] The microsomal fractions were obtained in a Beckman Spinco L-2 centrifuge at 150,000 *g* in 0.3 *M* sucrose with 10 μ*M* histidine, pH 7.45, after separation of residues and mitochondria (20 min 800 *g*; 10 min 6000 *g*; 20 min 20,000 *g*).

Riassunto. In numero di iodio dei grassi estratti da muscoli denervati è diminuito e il contenuto di colesterolo risulta aumentato. L'aumento è assai spiccato se si confrontano gli estratti lipidici delle frazioni microsomali.

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Effect of 2,4-Dinitrophenol on the Phosphorylases System of the Skeletal Muscle in vivo

The system phosphorylase A/phosphorylase B has an important role in the regulation of glycogenolysis in muscle. Evidence has been accumulating that phosphorylase A is a rate-limiting enzymic step in that metabolic pathway. Thus glycogenolytic agents as adrenalin, glucagon[1] and electrical stimulus[2], for instance, increase the breakdown of glycogen in muscle and accumulate hexosemonophosphates and lactic acid, with concomitant increasing of phosphorylase A. Among the glycogenolytic agents, 2,4-dinitrophenol (DNP) has been considered an exception[3,4].

The phosphorylases are inhibited by the drug in experiments either of glycogen phosphorolysis or of glycogen synthesis from glucose-1-phosphate, performed

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