(10). This suggests that the effect of carbohydrate metabolism on protein synthesis may not require insulin specifically, but that insulin increases carbohydrate metabolism and also probably stimulates protein synthesis directly. Evidence that insulin directly stimulates protein synthesis from ^{14}C-amino acids has been presented previously, puromycin being shown to inhibit protein synthesis (10).

Insulin stimulation of fat cell protein synthesis in the absence of glucose has not been reported with intact rat fat pad preparations (6–9), but only in the isolated fat cell preparation (10). The method of protein determination used with the isolated fat cells is probably more sensitive to small changes in protein synthesis than the extraction techniques utilized with intact fat pads and may, in part, explain this discrepancy. In all other respects studied, the metabolic activity of isolated fat cells and of the intact fat pad would appear to be similar.

Summary. Effects were studied of insulin and of varying the concentration of glucose upon L-leucine-^{14}C (UL) metabolism by isolated rat epididymal adipose tissue cells. At lower glucose concentrations, insulin increased CO_2 formation and lipid synthesis from labelled L-leucine. At higher glucose concentrations insulin decreased apparent CO_2 formation and lipid synthesis. Insulin stimulated protein synthesis at all levels of glucose concentration studied, but the increment was less at higher glucose concentrations.

1. Sinex, F. J., MacMillan, J., and Harting, A. B., J. Biol. Chem. **198**, 615 (1952).

2. Wool, I. G. and Krahl, M. E., Am. J. Physiol. **196**, 961 (1959).

3. Manchester, K. L. and Young, F. G., Biochem. J. **70**, 353 (1958).

4. Krahl, M. E., J. Biol. Chem. **200**, 99 (1953).

5. Herrera, M. G. and Renold, A. E., Biochim. Biophys. Acta **44**, 165 (1960).

6. Carruthers, B. M. and Winegrad, A. I., Am. J. Physiol. **202**, 605 (1962).

7. Krahl, M. E., Biochim. Biophys. Acta **35**, 556 (1959).

8. Krahl, M. E., Am. J. Physiol., **206**, 618 (1964).

9. Rodbell, M., J. Biol. Chem., **239**, 375 (1964).

10. Miller, L. and Beigelman, P. M., Biochem. Med. **1**, 129 (1967).

11. Stock, M. and Beigelman, P. M., Metabolism **16**, 1158 (1967).

12. Mans, R. J. and Novelli, R. G., Arch. Biochem. Biophys. **94**, 48 (1961).

13. Goodman, M. H., Am. J. Physiol. **206**, 129 (1964).

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Effects of Methylprednisolone on Plasma Lipids and Aortic Mucopolysaccharides of Normal and Cholesterol-Fed Rabbits* (33383)

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The administration of glucocorticoids to cholesterol-fed rabbits markedly reduces the incidence and severity of aortic atherosclerotic lesions despite a marked elevation in the plasma levels of cholesterol and triglycerides (1–3). Adlersberg *et al.* (4) attributed the protective effect of cortisone to a decreased permeability of the arterial wall resulting from alterations in the ground substance of

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TABLE I. The Effect of Daily Doses (4 mg) of Methylprednisolone (Medrol) on Plasma Total Cholesterol and Triglyceride Concentrations in Normal and Cholesterol-Fed Rabbits.

Group	Mean body wt. (kg)	Mean liver wt. (g)	Mean spleen wt. (g)	Plasma total cholesterol (C) (mg/100 ml)	Plasma triglycerides (TG) (mg/100 ml)	C/TG
Control	2.9 ± 0.3[a]	79 ± 7	1.4 ± 0.2	63 ± 12	88 ± 13	0.8
Medrol	2.5 ± 0.2	154 ± 9	1.7 ± 0.3	660 ± 162	1733 ±235	0.3
Cholesterol (1% in diet)	3.3 ± 0.2	132 ±26	3.8 ± 0.4	1800 ± 20	377 ± 43	4.9
Cholesterol + Medrol	2.5 ± 0.3	158 ± 3	8.0 ± 1.5	4300 ± 350	2667 ± 35	1.6

[a] ± SE.

the aorta. Since Fisher and Tapper (5) actually observed a decrease in the histochemically detectable acid mucopolysaccharides (AMPS) in the aortas of rabbits fed cholesterol and treated with cortisone, a study was undertaken to determine which, if any, of the major classes of aortic AMPS are affected.

Materials and Methods. Twelve adult male rabbits (New Zealand white strain) weighing 2–3 kg were divided into two groups of six animals each. One group was fed Purina rabbit chow and the other received the same diet containing 1% cholesterol (10 g of cholesterol in 75 ml of corn oil/kg of chow). The same amount of corn oil was added to the control chow diet. Food intake for each animal was restricted to 150 g/day (which was completely consumed by each rabbit) in order to minimize any differences in food intake that might arise with *ad libitum* feeding. Three animals in each group were given daily intramuscular injections of 4 mg of methylprednisolone[2] (Solu-Medrol, Medrol, The Upjohn Co., Kalamazoo, Mich.) during the two month duration of the experiment. Medrol was selected because of its reportedly low mineralocorticoid activity. Prior to sacrifice, the animals were fasted for 12 hr, and blood samples were taken by cardiac puncture. The blood was collected in heparinized tubes and the separated plasma was analyzed for total cholesterol and triglyceride concentrations (6, 7). The animals were then sacrificed by means of an intrathoracic injection of Somlethal (sodium pentobarbital). The aortas were quickly excised, and adherent blood and fat were removed and the adventitia was carefully stripped from the arteries. Tissue samples from the midsection of the aortic arch and from the thoracic region of the aorta were fixed in neutral formalin and the remainder of the artery was frozen and stored at −20° to await chemical analysis. The livers and spleens were weighed and sections of these tissues together with sections taken from lung and kidney were also fixed in neutral formalin. The tissues were imbedded in paraffin and stained with hematoxylin and eosin. Frozen sections of the unimbedded tissues were also prepared and stained with Sudan IV in order to show the presence or absence of lipids in these tissues.

The acid mucopolysaccharide (AMPS) composition of each aorta was determined by a modification of the procedure of Schiller *et al.* (8, 9). In this procedure, the aortas were defatted in a Soxhlet extraction apparatus with chloroform–methanol (2:1) and then hydrolyzed with papain. The papain digest (approximately 5 ml) was applied to a column (1 × 15 cm) of Dowex 1 (Bio-Rad AG-1 × 2, Calbiochem.) and the AMPS were eluted by a sodium chloride concentration gradient. The eluate was analyzed for hexuronic acid by the carbazole method (10) using D-glucuronolactone as a standard, and the results are expressed as μg of D-glucuronolactone/100 mg of dry, defatted tissues. The aortic AMPS were thus separated into four components: (i) hyaluronic acid (HA), eluted in 0.5 *M* NaCl, (ii) an unidentified AMPS, eluted in 0.8 *M* NaCl, (iii) heparitin sulfate (HS), eluted in 1.25 *M* NaCl, and (iv) the chondroitin sulfates (CS), eluted in 2.5 *M* NaCl.

Results. The administration of Medrol to

[2] The Medrol preparation was generously supplied by Dr. Samuel E. Stubbs of The Upjohn Co.

rabbits on a cholesterol-free diet induced very large increases in both plasma cholesterol (C) and plasma triglyceride (TG) concentration (Table I), although the C/TG ratio was somewhat reduced. Cholesterol feeding produced an elevated plasma cholesterol concentration but had a much smaller effect on the levels of TG. Medrol markedly enhanced the hypercholesterolemia induced by the cholesterol diet and produced a large increase in the TG levels. These animals had a combined plasma cholesterol and TG concentration of approximately 7 g/100 ml. However, Medrol had the effect of reducing the C/TG ratio in the animals fed cholesterol. Despite these large increases in plasma cholesterol and triglyceride concentrations, no atherosclerotic lesions were observed in the aortas of Medrol-treated animals. On the other hand, severe fatty lesions were seen (mainly in the arch) in the aortas of every cholesterol-fed rabbit nor receiving the hormone.

All of the animals fed cholesterol and treated with Medrol exhibited a severe fatty infiltration of the spleen. Microscopic examination of the spleens taken from cholesterol-fed animals not receiving Medrol revealed a slight lipid accumulation but the spleens of every animal given the cholesterol-free diet appeared normal. Splenomegaly was observed in every animal fed cholesterol but was more pronounced in the Medrol-treated group (Table I). Every Medrol-treated rabbit developed hepatomegaly and every cholesterol-fed animal had a fatty lever as revealed by visual and microscopic examination. Microscopic examination of the lungs of both hormone-treated and untreated animals fed cholesterol revealed definite atherosclerotic lesions in the pulmonary arteries.

In normal rabbit aortas, the chondroitin sulfates were present in the highest concentration followed in order of decreasing concentration by HS, the 0.8 *M* NaCl fraction and HA (Table II). Cholesterol feeding significantly lowered the levels of HA and the 0.8 *M* NaCl fraction but had no effect upon the other AMPS fractions or on the total concentration of AMPS. The administration of Medrol to animals given the regular chow

TABLE II. The Effect of Medrol on the Aortic Acid Mucopolysaccharide Composition of Control and Cholesterol-Fed Rabbits.*

Group	No. of animals	Glucuronolactone (μg/100 mg of dry, defatted aorta; mean ± SE)								
		HA		0.8 *M* NaCl		HS		CS		Total	
A. Control	3	48 ± 5	<.01	83 ± 9	<.05	93 ± 6	<.005	133 ± 8	<.05	368 ± 24	<.05
B. Control + Medrol (4 mg) daily	3	32 ± 3		76 ± 9		92 ± 15		154 ± 31		353 ± 62	
C. Cholesterol (1%)	3	33 ± 6	<.02	64 ± 7	<.005	87 ± 8	<.05	135 ± 6		321 ± 24	
D. Cholesterol (1%) + Medrol	3	55 ± 10		106 ± 10		86 ± 11		162 ± 13		408 ± 37	

* The values appearing in the braces are *p* values obtained by Student's *t* test for significance. Similar comparisons made between other groups of data show that the values are not significantly different from one another.

diet decreased only the concentration of HA, but Medrol and cholesterol together caused a marked increase in the 0.8 *M* NaCl fraction and a lesser increase in the CS fraction. A comparison of the data from Medrol-treated animals fed chow diet with the data from Medrol-treated animals receiving cholesterol revealed differences in only the HA and 0.8 *M* NaCl fractions. However, a comparison of the data from the latter group with those from the cholesterol-fed animals not receiving Medrol revealed an increase in all AMPS fractions except HS.

Discussion. In the present study, it was clearly demonstrated that a high dose level of Medrol inhibits the development of atherosclerotic lesions in the aortas of rabbits fed cholesterol despite a marked hyperlipemia. Associated with this protective effect was an alteration in the acid mucopolysaccharide composition of the intima–media of the aorta. The very high plasma levels of cholesterol and triglycerides were probably responsible for the pronounced lipid infiltration observed in the spleens of these animals.

There is evidence that the protective effect of glucocorticoids may be due to changes in both the circulating lipids and the AMPS composition of the aorta. Moss and Drury (11) demonstrated that cortisone can alter the flotation pattern of circulating lipids by elevating the concentrations of the S_f 12–400 lipoproteins and it was suggested that this may shift the circulating cholesterol into a relatively nonatherogenic lipoprotein fraction. Adlersberg *et al.* (4) found that the administration of hyaluronidase to rabbits fed cholesterol partially abolished the antiatherogenic action of cortisone, and they postulated that the ground substance of the aorta was involved in the protective effect of the hormone, possibly through an alteration in the permeability of the aortic wall to circulating lipids. In the present study, atherosclerotic lesions were observed in the pulmonary arteries of cholesterol-fed rabbits whether or not they were treated with Medrol. Therefore, connective tissue changes in the aorta may be more important than plasma lipid changes in the protective effect of Medrol since it is unlikely that lipid differences exist between the blood passing through the lungs and that coming in contact with the aortic wall.

Fisher and Tapper (5) found that cortisone decreased the metachromatic staining properties of aortas from cholesterol-fed rabbits, but in the present study an increase rather than a decrease in the total concentration of aortic AMPS was produced by Medrol treatment. This increase was due largely to the 0.8 *M* NaCl fraction which contains AMPS of relatively low sulfate content (9). Since metachromatic staining is related to the degree of sulfation of the AMPS, an increase in the 0.8 *M* NaCl fraction might result in a decreased metachromatic staining of the tissue. This staining property of the aorta may also be influenced by the amount of interaction between AMPS and connective tissue proteins and hence is not always a reliable index of the AMPS content of the tissue.

Except for its effect upon hyaluronic acid, Medrol did not significantly alter the aortic AMPS composition in animals fed the regular chow diet. Yet it produced significant changes when cholesterol was fed and actually reversed the aortic AMPS effects usually seen when cholesterol alone is fed. Forman *et al.* (12) found that prolonged cholesterol feeding stimulated the synthesis of sulfated AMPS in aortas, and Thomas (13) showed that cortisone inhibits the degradation of AMPS by lysosomal enzymes, presumably by the stabilization of the lysosomal membranes. It has been shown by others (14) that cortisone produces abnormal inflammatory and reparative reactions following aortic damage. Therefore, it is possible that Medrol interferes with the sulfation of chondroitin sulfate, the synthesis of which may have been stimulated by prolonged cholesterol feeding, and it may have also inhibited the normal degradation of CS. This could explain the accumulation of AMPS of low sulfate content (the 0.8 *M* NaCl fraction) and the accumulation of chondroitin sulfates.

Summary. Methylprednisolone (Medrol) was administered to normal and cholesterol-fed rabbits for a period of 2 months, and its effects upon plasma cholesterol and triglyceride concentrations and upon the content of

aortic acid mucopolysaccharides (AMPS) were studied. Rabbits fed a chow diet developed both a hypercholesterolemia and a hypertriglyceridemia in response to Medrol, and the already elevated cholesterol and triglyceride levels induced by cholesterol feeding were further elevated by Medrol. Despite the hyperlipemia, none of the Medrol-treated animals developed aortic atherosclerosis. Medrol had no significant effect upon the total aortic AMPS content in control animals but it did alter the aortic AMPS content and pattern of cholesterol-fed rabbits. The most significant effect was an increase in an AMPS of low sulfate content. Since all animals fed cholesterol developed some degree of pulmonary atherosclerosis, it is postulated that the antiatherogenic action of the hormone on the aorta is more closely related to alterations in the AMPS pattern than to changes in the pattern of circulating lipids.

1. Bailey, J. M. and Butler, J., Nature **212**, 731 (1966).

2. Wang, C., Schaefer, L. E., and Adlersberg, D., Endocrinology **56**, 628 (1955).

3. Forbes, J. C., Higgins, E. S., and Petterson, O. M., Metabolism **11**, 1232 (1962).

4. Adlersberg, D., Wang, C., and Strauss, L., J. Mt. Sinai Hosp., N. Y., **24**, 655 (1957).

5. Fisher, E. R. and Tapper, E., Am. J. Pathol **37**, 713 (1960).

6. Block, W. D., Jarrett, K. J., Jr., and Levine, J. B., *in* "Technicon Symposia," p. 345. Technicon, Ardsley, New York (1965).

7. Kessler, G. and Leder, H. *in* "Technicon Symposia," p. 341. Technicon, Ardsley, New York (1965).

8. Schiller, S., Slover, G. A., and Dorfman, A., J. Biol. Chem. **236**, 983 (1961).

9. Alper, R., Prior, J. T., and Ruegamer, W. R., J. Atherosclerosis Res., in press.

10. Dische, Z., J. Biol. Chem. **167**, 189 (1947).

11. Moss, L. D. and Drury, A., J. Mt. Sinai Hosp., N. Y. **24**, 1047 (1957).

12. Forman, D. T., Choi, S. S., and Taylor, C. B., Arch. Pathol. **85**, 80 (1968).

13. Thomas, L., Biophys. J., Suppl., 207 (1964).

14. Prior, J. T., Rohner, R. F., Camp, F. A., and Rustad, H., Arch. Pathol. **67**, 159 (1959).

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Method for Increasing the Diagnostic Capacity of the Complement Fixation Test in Some Respiratory Virus Infections (33384)

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It has been established, that the complement fixation (CF) test is of limited value for the diagnosis of respiratory virus infection in infancy and early childhood (1, 2). In both adeno- and parainfluenza virus infections, and especially in respiratory syncytial (RS) virus infection, not all of the diseased children from whom virus can be isolated, develop a significant antibody response as revealed by the CF test.

This might be explained in part by the fact that the 19 S (γM) immunoglobulin which is detected as the first antibody in many primary virus infections has little complement-fixing activity (3).

It was shown by Grubb (4) and Jones (5) that the polysaccharide dextran (6) reveals incomplete rhesus antibody when added in certain concentrations to the mixture of erythrocytes and serum. It had been shown some years earlier that bovine serum albumin is also a valuable diluent for rhesus typing reagents (7).

With the purpose of increasing the sensitivity of the diagnostic CF test for respiratory infections, the mixture of antigen, test serum, and complement has been supplemented with dextran and incubated overnight before the addition of the hemolytic system.

The CF titers obtained in tests performed with different dextran concentrations are reported in the present paper. Different respi-