Adrenal Cortex, Lipid Metabolism, and Atherosclerosis: Experimental Studies in the Rabbit*

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Previously reported observations revealed elevations of serum lipid fractions in patients treated with prolonged courses and large doses of cortisone and/or corticotropin (*1–3*). Not infrequently the serums of these patients had a milky (opaque) appearance. These observations have been extended to experimental animals (*4–6*).

Of a group of 150 rabbits on a diet of Purina chow that received cortisone acetate, hydrocortisone acetate, or corticotropin, 41 survived for 10 days or longer. The effect of the hormones on the plasma lipid partition is presented in rows 2–4, Table 1. The greatest alteration occurred in the neutral fat fraction. The most opaque plasmas contained the highest levels of neutral fat. The changes in total and esterified plasma cholesterol and of phospholipid were less pronounced than those of neutral fat.

These studies were extended to the cholesterol-fed rabbit. The technique of administering pure cholesterol to the rabbit without using fat solvents or stomach tube, and the effects of cholesterol feeding on the plasma lipid partition of normal young rabbits have been reported (*7*). These data are summarized in row 5, Table 1. The results of cholesterol feeding combined with daily injections of cortisone acetate, hydrocortisone acetate, or corticotropin are presented in rows 6–8, Table 1.

It is evident that the combination of cortisone or hydrocortisone injections with cholesterol feeding results in considerable enhancement of the elevations of all plasma lipid fractions observed with cholesterol feeding alone. Serum cholesterol levels as high as 5050 mg/100 ml were occasionally observed, which, to our knowledge, have not been produced previously by any experimental procedure. Discontinuation of corticosteroid administration but continuation of cholesterol feeding was followed by a return of the plasma lipids to levels characteristic for cholesterol feeding alone. However, when corticotropin injections were combined with cholesterol feeding, no significant enhancement was observed.

In all the rabbits, careful pathological studies were performed. After 1 mo of cholesterol feeding, the rabbits developed definite signs of atherosclerosis of the ascending aorta, of the arch, of the mitral and/or aortic valve, and only occasionally of the pulmonary and coronary arteries. After 3 to 4 mo, large, confluent plaques covered the ascending and descending aorta, the aortic and mitral valves, the coronary and pulmonary arteries. In addition, lipidosis of spleen, kidneys, joints, and skin was frequently present. Animals subjected to cortisone or hydrocortisone injections in addition to the cholesterol feeding exhibited, at comparable time intervals, marked inhibition of atherogenesis and delayed deposition of cholesterol in the tissues when compared with those treated with cholesterol alone.

Recently Seifter *et al.* (*8*) noted, in cholesterol-fed rabbits injected with hyaluronidase, enhancement of atherogenesis, despite lower plasma cholesterol levels; these effects were attributed to increased tissue permeability. Our studies suggest that corticosteroids diminish tissue permeability and that their administration results in diminished atherogenesis, despite higher plasma cholesterol levels. Additional studies utilizing combinations of cholesterol, corticosteroids, and hyaluronidase support this concept (*9*).

In summary then, cortisone and hydrocortisone produce significant, although moderate, elevations of the plasma lipid fractions in the rabbit. The combination of cholesterol feeding with daily injections of these hormones produces extreme elevations of all plasma lipid fractions, especially of plasma cholesterol. Nevertheless, atherogenesis and deposition of cholesterol in other tissues, in the latter group, is definitely depressed, possibly because of the diminished tissue permeability produced by these hormones.

Table 1. Average maximal changes in plasma lipids observed with different regimens of treatment in the rabbit.

	No. of animals	Cholesterol		Phospholipids		Neutral fats		Total lipids		Time (wk)
		(mg %)	Times normal	(mg %)	Times normal	(mg %)	Times normal	(mg %)	Times normal	
Control	119	49		103		198		350		
Corticotropin	15	95	1.9	150	1.5	337	1.7	582	1.7	5.5
Hydrocortisone	8	93	1.9	187	1.8	692	3.5	972	2.8	3
Cortisone	18	158	3.1	278	2.8	1019	5.0	1454	4.2	5
Cholesterol	28	1476	30.0	460	4.6	1094	5.5	3030	8.7	12
Cholesterol and corticotropin	4	877	17.9	423	4.2	955	4.8	2255	6.4	7
Cholesterol and hydrocortisone	4	1745	35.7	745	7.5	2260	11.3	4750	13.6	5
Cholesterol and cortisone	18	2079	42.5	890	8.9	1736	8.8	4705	13.4	9

References and Notes

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Primary Isolation and Propagation of Influenza Virus in Cultures of Human Embryonic Renal Tissue*

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A virus of the influenza-A type has been isolated directly from the nasal washings of a patient by single passage in cultures of human embryonic renal tissue and has been propagated by additional passages in cultures of this same tissue. The primary isolation of influenza virus by tissue culture from which hemagglutinins can be demonstrated and utilized for serological identification of the virus has not been previously reported, nor has growth of influenza virus been demonstrated in cultures of human tissue. Moreover, this use of human tissue culture provides an opportunity to study the characteristics of influenza virus without the necessity of passage through cells of another host species.

Kidneys from a 25-cm human embryo obtained following a spontaneous abortion were preserved as small fragments, 1 to 2 mm in diameter, in Hanks-Simms solution (*1*) for 13 days at 4°C prior to preparation of the cultures; 15 to 20 fragments were planted on the walls of 50-ml glass tubes that had been preheated to 45°C (*2*). To each tube was added 3 ml of nutrient medium: bovine amniotic fluid, 90 percent; bovine embryo extract, 5 percent; inactivated horse serum, 5 percent; phenol red 0.002 percent; penicillin, 200 units/ml, and streptomycin, 200 μg/ml (*3*). The cultures were incubated at 35°C in a horizontal stationary position, and nutrient fluids were replaced at weekly intervals. By the seventh day there was epithelial outgrowth from most of the fragments, and this had attained a diameter of 0.5 to 1.0 cm at 14 days. Each culture was inoculated at this time with 0.1 ml of nasal washing obtained from a patient in Jan. 1953. Nutrient fluids were replaced twice weekly following inoculation.

The presence of virus was determined by hemagglutination at room temperature both with 0.25 percent human type-O erythrocytes and pooled fowl erythrocytes and also by growth in subsequent tissue culture and in the amniotic sac of 10- to 11-day chick embryos. Hemagglutination-inhibition tests using human type-O erythrocytes were carried out with standard influenza virus-immune rooster antiserums and hemagglutinating antigen, strains PR8, FM1, FW-1-50 and Lee, obtained through the Biological Depot, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D.C. All antiserums were treated with receptor-destroying enzyme prepared by culture filtrates of the Inaba strain of *Vibrio cholerae*.

Table 1 shows the hemagglutination titers of tissue-culture fluids as determined by the addition of 0.25 percent human type-O erythrocytes. Although hemagglutinins could be detected earlier at the second tissue-culture passage, there was no increase in titer. The lack of hemagglutination by subsequent fluids was probably due to a paucity of additional susceptible cells for viral multiplication. Treatment of these fluids with receptor-destroying enzyme of *Vibrio cholerae* did not increase the hemagglutinating titers, although fresh nutrient mediums had sufficient nonspecific inhibitor to prevent hemagglutination of human type-O red blood cells by 16 hemagglutinating units of infectious PR8 virus after overnight incubation at 37°C. Infectivity titrations in the amniotic sac of the chick embryo demonstrated an increase from $10^{2.6}$ EID_{50} in the nasal washing to $10^{5.0}$ EID_{50} in the pooled third-day fluids from the second tissue-culture passage. It is likely that these titers were an index of the number of viral particles capable of adaptation to the chick embryo rather than a measure of the total infectious virus.

In no instance were hemagglutinins for fowl erythrocytes demonstrated in tissue-culture fluids. However, the initial nasal washing and each tissue-culture fluid shown in Table 1 upon single passage through the amniotic sac of the chick embryo yielded hemagglutinins for fowl erythrocytes. Inoculation of these same tissue-culture fluids in the allantoic sac of the chick embryo did not result in viral growth as measured by hemagglutination. The amount of virus inhibited by nonspecific inhibitor of hemagglutination

Table 1. Hemagglutination titers of fluids from individual tissue cultures, expressed as the reciprocal of final virus dilution. The four cultures *a, b, c, d* were inoculated with 0.1 ml of original nasal washing; at second passage four more cultures, *w, x, y, z*, were inoculated with 0.1 ml of the third-day fluid from culture *c*.

Day	First tissue culture passage				Second tissue-culture passage			
	a	*b*	*c*	*d*	*w*	*x*	*y*	*z*
0	0	0	0	0	0	0	0	0
3	0	0	64	0	128	32	32	64
7	0	32	8	32	0	0	0	0
10	0	0	0	0	0	0	0	0