tor VII-X production by liver slices was studied because it was found that plasma Factor VII-X levels were significantly elevated in rats fed atherogenic diets. Although unexplained variations occurred, in 15 out of 18 paired experiments the production of Factor VII-X during incubation was greater in flasks containing liver slices from test animals than from controls. Expressed as a ratio (test:control) the median value of Factor VII-X production per mg protein in the flask was 1.9. Thus there appeared to be increased synthesis of Factor VII-X in flasks from test rats though the possibility of delayed degradation was not excluded.

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Effects of Triparanol on the Hyperlipemia of Experimental Nephrosis.* (29945)

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We(1-5) have repeatedly observed and the literature convincingly demonstrates that hyperlipemia and hypercholesteremia of varying degrees develop along with hypoproteinemia during the development of nephrosis. This is true both of clinical nephrosis in patients and of nephrosis produced experimentally in dogs and rats by administration of antikidney serum. This inverse relationship between the occurrence of hypoproteinemia and the occurrence of hyperlipemia was so consistent that we decided to study the effects of an antilipemic agent, triparanol (MER-29), which is known to arrest cholesterol synthesis at the desmosterol stage. We wondered whether arrest of cholesterol synthesis and depression of the hyperlipemia would have any effect on the loss of plasma proteins and the resulting hypoproteinemia.

Background information. A survey of the literature revealed elaborate studies on the relationship of hypoproteinemia and hyperlipemia in nephrosis without any definite consensus to pinpoint that relationship etiologically or consequentially.

The nephrotic syndrome, whether experimentally produced or clinically encountered, is not a disease but a complex of clinical signs and symptoms and a definite set of chemical findings in the blood and urine that are sometimes encountered in other disease entities. The salient features characterizing this syndrome are massive proteinuria, hypoproteinemia, hypoalbuminemia, hyperlipemia, hypercholesteremia and usually edema.

The site of the pathologic changes in the nephrotic syndrome is the kidney generally and the glomerular basement membrane specifically. Thickening of the glomerular base-

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ment membrane and fusion of the foot processes are the chief characteristics of the lesion. Increased permeability of the glomerular membrane to proteins leads to excessive loss of plasma proteins into the urine and to the subsequent hypoproteinemia. Along with the severe decrease in plasma proteins a distortion of the lipid-transport mechanism occurs and leads to increases of plasma cholesterol, triglycerides and other lipids.

Even though the loss of protein into the urine is huge, proteinuria cannot be considered the sole cause of hypoproteinemia in nephrosis.

Several mechanisms combine to exaggerate the severe decrease of plasma protein, especially the hypoalbuminemia. Among these are (1) the huge loss of plasma proteins into the urine, (2) increased catabolism of plasma proteins, and (3) impaired appetite, low protein intake and malnutrition. The severe hypoproteinemia, specifically hypoalbuminemia, occurs in the nephrotic syndrome despite increased synthesis of plasma proteins.

Lipid abnormalities culminating in hyperlipemia in nephrosis may be associated with the hypoalbuminemia, as pointed out by Saffran and Kalant(6). Rosenman and associates(7,8) produced experimental nephrosis in rats by giving antikidney serum and demonstrated the development of a syndrome like the human nephrotic syndrome; the serum lipids(8) increased markedly from an endogenous origin but increasing dietary lipids intensified the hyperlipemia(7). According to these workers, the increase of blood cholesterol is not due to an earlier alteration in intestinal absorption or excretion(9) or to the rate at which it is produced by the liver(10). They(11) believed they showed that if urinary loss of albumin was blocked by ligation of the rat's ureters or by ureter-vena cava anastomosis before injection of nephrotoxic serum, no increase in serum cholesterol or total lipid occurred after injection of the serum and the appearance of all the other features of the disease. Infusion of concentrates of serum albumin into nephrotic rats prevented or reduced the hyperlipemia with the increase in concentration of serum albumin. They concluded that increase of serum lipids in nephrotic rats was due to loss of certain substances from the plasma, one of which was albumin. This was confirmed by Baxter and colleagues(12,13) in patients in whom the increased concentration of serum lipids fell after administration of albumin infusions that increased plasma albumin to normal.

Svanborg(14) presented an attractive hypothesis to explain the relationship between hypoalbuminemia and hyperlipemia coexisting in the nephrotic syndrome. He suggested that to satisfy its energy needs, the body relies heavily on nonesterified fatty acids, and these fatty acids are transported only by serum albumin. In the presence of severe hypoalbuminemia, therefore, there will be either metabolic starvation or a recourse to some other mechanism for supply of energy. The beta-lipoproteins carry large amounts of triglycerides which could be released to supply energy. Marsh and Drabkin(15) reported that restoration of plasma proteins to normal levels will also restore lipids to normal levels. This is not well substantiated and evidence to the contrary is available. Furthermore, the situation is not that simple. As mentioned above, there are a number of clinical entities in which hypoalbuminemia is present alone and not associated with hyperlipemia. Also there is evidence that hyperlipemia precedes hypoalbuminemia.

Consequent to the hypoproteinemia and hypoalbuminemia and the increased reabsorption of sodium, edema develops. The edema leads to hypovolemia, liberation of antidiuretic hormone and aldosterone increases, and the vicious cycle continues. The massive retention of fluid and the edema are due to hypoproteinemia, hypovolemia, increased secretion of aldosterone and increased production of antidiuretic hormone.

Methods. Experimental nephrosis was produced in dogs by intravenous administration of serum obtained from rabbits sensitized against dog kidney. The kidney homogenate was prepared and injected into the rabbits intraperitoneally as described previously(5). Healthy young dogs weighing between 5 and 10 kg were housed individually in separate metabolism cages and given a standard diet consisting of one pound of Hill's dog food per day, which constituted a well-balanced diet.

TABLE I. Effects of Triparanol on Changes Induced in Serum Cholesterol by Nephrotoxic Serum (NTS).

Cholesterol (mg/100 ml of serum)					
NTS only			NTS + triparanol		
Control period (avg)	Experimental period (max.)	Diff.	Control period (avg)	Experimental period (max.)	Diff.
176	580	404	160	310	150
204	354	150	165	210	45
128	320	192	235	225	−10
163	368	205	128	270	142
174	394	220	228	254	26
Mean difference		234 ± 44			71 ± 30

For production of nephrosis, the dogs were divided into 2 groups. Group 1 was given rabbit antikidney serum (NTS) intravenously in amounts of 4.4 ml/kg divided into 9 doses and administered 3 times a day for 3 days. Group 2 was given the same dosage of nephrotoxic serum as Group 1 but also was given triparanol by mouth, 25 mg/kg of body weight daily, for several days before, during, and for 2 weeks after administration of NTS.

Urine was collected in 24-hour periods and total volume, specific gravity, osmolality, and protein content were determined throughout the period of study. Blood samples were drawn from the jugular vein at specified intervals. Chemical and electrophoretic studies were made on blood and urine to determine proteins, lipoproteins, glycoproteins, cholesterol and total lipids before, during and for long periods after administration of NTS alone and of NTS plus triparanol. The various chemical procedures used have been described(5). For estimation of total lipid, the Bloor(16) procedure was used. Total proteins of serum and urine were determined by the Kingsley biuret method.

Specimens of the kidneys were studied with light and electron microscopes before and at intervals after administration of the various agents and also at the end of the investigation. For light microscopy, all sections from the kidneys were fixed in Formalin and stained with hematoxylin and eosin. The sections were examined and the lesions were described and recorded without knowledge of the experimental procedure that had been carried out on the animals.

Results. Blood chemical changes. As described previously(5), in the dogs receiving NTS, serum lipids and serum cholesterol definitely increased, total serum proteins markedly decreased, and proteinuria of variable severity developed, reaching a peak before the tenth day after administration of NTS. The changes in electrophoretic patterns of the serum proteins, lipoproteins and glycoproteins were not much different from those reported previously(5).

Table I presents changes in serum cholesterol induced by NTS alone and the effects of triparanol on these changes. In the dogs receiving NTS alone, serum cholesterol increased, on the average, from 169 mg/100 ml in the control period to 403 mg at the peak of effect of NTS. Thus the increase due to NTS averaged 234 mg. In the dogs receiving NTS plus triparanol, values for serum cholesterol averaged 183 mg in the control period and 254 mg at the peak of effect of NTS plus triparanol, giving an average increase of 71 mg, which is significantly less than the corresponding figure of 234 mg observed in the group receiving NTS alone.

Table II presents changes in total serum lipids. It is evident that NTS caused a definite increase in total lipids and that the effect of triparanol on the hyperlipemia was insignificant. Under the influence of NTS alone the maximal increase in serum lipids averaged 280 mg per 100 ml; the increase was from 592 mg in the control period to 872 mg at the peak of effect of NTS. When NTS was given with triparanol the increase in serum lipids was slightly but insignificantly less than with NTS alone; values for lipids averaged 519 mg in the control period and 719 mg after treatment with NTS plus triparanol, making the average difference 201 mg.

Table III presents changes in concentration of serum and urinary proteins and in

TABLE II. Effects of Triparanol on Changes Induced in Total Lipids by Nephrotoxic Serum (NTS).

Total lipids (mg/100 ml of serum)					
NTS only			NTS + triparanol		
Control period (avg)	Experimental period (max.)	Diff.	Control period (avg)	Experimental period (max.)	Diff.
550	1000	450	624	850	226
600	970	370	425	643	218
545	825	280	415	526	111
640	735	95	475	758	283
625	830	205	654	820	166
Mean difference		280 ± 55			201 ± 29

TABLE III. Effects of Triparanol on Changes Induced in Serum and Urinary Protein and in Blood Urea by Nephrotoxic Serum (NTS).

Lowest serum protein during exp. period (g/100 ml)			Maximal proteinuria during exp. period (g/100 ml)			Maximal blood urea during exp period (mg/100 ml)		
NTS only	NTS + triparanol	Diff.	NTS only	NTS + triparanol	Diff.	NTS only	NTS + triparanol	Diff.
3.3	3.1	− .2	2.5	2.6	.1	59	53	− 6
3.5	2.8	− .7	6.0	4.3	−1.7	38	84	46
3.6	3.4	− .2	2.8	2.8	0	32	56	24
4.7	3.5	−1.2	3.5	2.6	− .9	60	49	−11
4.6	4.8	.2	2.2	2.2	0	42	37	− 5
Mean difference		− .4			− .05			9.6

concentration of blood urea. At the peak of effect, which occurred at about the fifth to tenth day, the value for total serum proteins decreased from an average of 5.7 g/100 ml, before NTS alone to an average of 3.9 g, and maximal value for urinary protein after NTS alone averaged 3.4 g/100 ml. In the dogs that received NTS plus triparanol the effects were not significantly different: the value for total serum proteins decreased from 5.8 g to 3.5 g and maximal value for urinary protein was 2.9 g. At the peak of effect the value for blood urea averaged 46.2 mg after NTS alone and 55.8 mg after NTS plus triparanol.

The histologic changes in the kidneys as seen with the light microscope were the same in the dogs receiving NTS plus triparanol as in those receiving NTS alone, namely endothelial proliferation in glomerular tufts, adhesions of tuft to capsule at several points, focal fibrosis of glomeruli, hyalinization of tufts with periglomerular fibrosis, and thickening of the basement membrane. The tubular epithelium showed hydropic degeneration.

Studies with the electron microscope revealed the following findings on the effects of NTS: The endothelial cells of the glomerular capillaries became separated from the basement membrane; this was associated with considerable swelling, decreased density and vesiculation of the cytoplasm. There was a definite increase in the number of elements of the endoplasmic reticulum including membrane-bound and free ribosomes. The basement membranes were thickened and the epithelial cells showed fusion of foot processes. Administration of triparanol with NTS had no effect on any of the changes in renal structure induced by NTS. The electron microscopic findings were the same as those described above for NTS alone.

In general, the dogs receiving NTS plus triparanol looked sicker than did those receiving NTS alone. Evidently, triparanol aggravated the course of the disease.

Summary. Since triparanol (MER-29) has been shown to arrest synthesis of cholesterol at the desmosterol stage and prevent an increase of serum cholesterol, we investigated whether this drug would decrease hypercholesteremia and hyperlipemia and reveal a relationship between increase of serum cholesterol and decrease of serum proteins in nephrosis. Experimental nephrosis was produced in dogs by intravenous administration of serum from rabbits sensitized to dog kid-

ney (NTS). Triparanol was given orally in doses of 25 mg/kg of body weight per day before, during, and for 2 weeks after administration of NTS. Total serum lipids, serum cholesterol, and serum and urinary proteins were determined before, during, and for several weeks after injection of NTS. Triparanol decreased serum cholesterol markedly and serum lipids slightly, but it had no influence on hypoproteinemia and proteinuria. The histologic changes in the kidneys were the same in the dogs receiving NTS plus triparanol as in the dogs receiving NTS alone, namely endothelial proliferation in glomerular tufts, adhesion of tuft to capsule at several points, focal fibrosis of glomeruli, hyalinization of tufts with periglomerular fibrosis, irregular thickening of basement membrane, and fusion of foot processes. The tubular epithelium showed hydropic degeneration. Triparanol aggravated the course of the disease.

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Incorporation of Plasma Cholesterol-4-C^{14} into Egg Yolk Cholesterol.* (29946)

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To date there is no direct evidence that cholesterol from the blood of the laying hen is transferred across the ovarian membranes into developing egg yolks. In previous work, laying hens have been given C^{14}-acetate or H^2-acetate. These labels have subsequently been found in all of the constituents of eggs, including the cholesterol of the yolk(1,2,3). However, because of the expected rapid conversion of the administered acetate to cholesterol in the liver and recycling of cholesterol into the blood, the finding of labeled cholesterol in the egg yolk could have been explained by at least two possible mechanisms. First, cholesterol of hepatic origin synthesized from the labeled acetate might have been transported *via* the blood into the yolk. Second, circulating labeled acetate might have been taken up by the ovary which then synthesized cholesterol and transferred it into the yolk. This latter hypothesis is

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