CHANGES IN LIPID CONTENT OF SERUM AND OF LIVER FOLLOWING BILATERAL RENAL ABLATION OR URETERAL LIGATION*

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Certain changes in the lipid content of the serum following bilateral nephrectomy or ureteral ligation have been noted (1, 2). The extent and character of these changes, the factors affecting them, and the associated changes in the composition of the liver form the subject of this paper. For comparative purposes the effects of unilateral nephrectomy, of splenectomy, of fasting, and of other procedures have also been studied.

Material and Methods

Twenty-three adult dogs and two *rhesus* monkeys (*Macaca mulatta*) were employed. For some days prior to operation the dogs were maintained on an ordinary dog chow ration, except in three instances, in which a low potassium diet was employed (3). The monkeys were maintained on the usual mixed laboratory ration. After bilateral nephrectomy or bilateral ureteral ligation no food or water was given, in order to minimize vomiting and consequent salt depletion (4). Otherwise food and water were given *ad libitum*, with a few exceptions which are noted in the tables. Animals were sacrificed before death occurred spontaneously.

Operations were performed under nembutal anesthesia with strict aseptic precautions. Liver specimens were obtained by biopsy of this organ. The specimen of liver obtained after nephrectomy or other procedure could therefore always be compared with a control sample of the liver of that same animal. All the difficulties which arise from a comparison with average normal values were thus avoided. Technically, the procedure was carried out without much bleeding by the use of a Bovie electrocautery knife. Any coagulated portions were cut away from the biopsy specimen before analysis. Blood was drawn under oil, allowed to clot, and the serum removed after centrifugation. By the chemical methods in use in this laboratory (5-9) cholesterol, unesterified cholesterol, fatty acids,¹ and lipid phosphorus were determined. Proteins were determined by the Kjeldahl method (10), since the falling

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¹ Fatty acids refer to the "titrated fatty acids" described in a previous paper not to the "total fatty acids" in earlier papers of this same series in which a correction factor was used which, in 1937, was shown to be at fault (6).

TABLE I
*Effects of Unilateral Nephrectomy, of Bilateral Nephrectomy, and of Splenectomy on the Serum
 Lipids and Protein of the Dog*

Dog No.	Days after first operation	Procedure*	Blood non-protein nitrogen	Serum					
				Total fatty acids	Lipid phosphorus	Cholesterol		Neutral† fat	Total protein
						Total	Esterified†		
			mg. per cent	mm per liter	mm per liter	mm per liter	mm per liter	mm per liter	gm. per cent
1§	0	Nephrectomy 1							
	10	Nephrectomy 2	33	13.8	4.3	5.2	(3.5)	(2.5)	7.30
	12		151	15.3	4.6	4.9	(3.3)	(3.7)	
	15		319	15.0	5.3	5.8	(3.9)	(1.6)	
	16		400	20.6	7.1	7.4	(5.0)	(2.8)	8.29
2	-3			9.1	3.6	3.3	(2.3)	(0.4)	5.52
	0	Nephrectomy 1	33	7.5	3.0	2.6	(1.8)	(0.3)	6.06
	4	Nephrectomy 2		8.3	2.7	2.9	(2.0)	(1.5)	
	5		90	11.6	3.6	3.9	(2.6)	(2.6)	5.71
	7		200	12.4	3.9	4.8	(3.2)	(2.2)	5.83
	9		345	14.6	4.4	6.5	(4.4)	(2.1)	6.62
3	0	Nephrectomy 1		9.8	3.4	3.4	(2.3)	(1.6)	4.90
	8			9.4	3.4	3.3	(2.2)	(1.0)	5.90
	15			10.6	3.9	3.3	(2.3)	(1.3)	5.74
	27	Nephrectomy 2	31	10.1	3.7	3.5	(2.4)	(1.1)	5.75
	29		135	12.2	4.3	4.5	(3.0)	(1.5)	5.54
	30		184	13.2	4.3	4.1	(2.8)	(2.5)	
	31		202	13.7	4.7	4.3	(2.9)	(2.4)	6.04
	32		247	14.3	4.6	6.3	(4.3)	(1.7)	6.46
4	0	Nephrectomy 1; liver biopsy		8.9		2.8	(1.9)		
	8	Nephrectomy 2							5.15
	9		58	9.6	3.5	3.6	2.5	(0.8)	
	11		254	12.5	4.5	4.8	3.6	(0.8)	7.37
	11		291	12.9		5.1	(3.5)		
5	0	Nephrectomy 1; liver biopsy		8.0	3.1	2.5	1.7	(0.6)	5.80
	3			9.4	3.2	3.1	2.1	(1.5)	6.00
	12			8.8	3.2	2.7	1.9	(1.3)	6.36
	38	Nephrectomy 2; liver biopsy	36	9.2	3.5	2.9	2.2	(0.8)	6.00
	40			15.4	5.2	5.1	3.5	(2.5)	7.42
	41		174	15.7	5.4	6.1	4.6	(1.5)	

* After blood of same date.

† Calculated values in parentheses.

§ Low potassium diet.

TABLE I—*Concluded*

Dog No.	Days after first operation	Procedure*	Blood non-protein nitrogen	Serum					
				Total fatty acids	Lipid phosphorus	Cholesterol		Neutral† fat	Total protein
						Total	Esterified‡		
			mg. per cent	mm per liter	mm per liter	mm per liter	mm per liter	mm per liter	gm. per cent
6	0	Nephrectomy 1; liver biopsy		9.9	3.9	3.4	2.4	(0.6)	6.00
	2			14.7	5.0	5.3	3.6	(2.3)	6.72
	4			11.6	4.0	4.7	3.3	(1.0)	6.01
	7	Nephrectomy 2 Glucose	21	10.1	3.8	3.9	2.7	(0.7)	5.58
	9		139	16.1	6.0	5.6	3.2	(2.0)	5.39
	11		251	17.2	5.7	7.3	4.9	(2.0)	5.09
7	0	Nephrectomy 1		10.5		3.2	(2.2)		
	4			10.6	3.7	3.6	(2.4)	(1.4)	5.47
	7	Nephrectomy 2; liver biopsy		9.6	3.6	3.5	(2.4)	(0.8)	5.46
	11	Glucose	311	18.0	5.9	6.6	(4.5)	(2.8)	5.42
8**	0	Nephrectomy 1		8.5	3.1	2.6	1.7	(1.2)	4.84
	3			12.4	3.9	4.4	2.8	(2.6)	5.38
	12			10.2	3.2	4.0	2.5	(1.9)	5.10
9	0	Splenectomy		10.2	3.2	2.9	2.1	(2.3)	5.46
	3			11.4	3.8	3.9	2.6	(1.9)	5.23
	7			8.7	3.0	2.9	(2.0)	(1.4)	4.98
	15	Nephrectomy 1		10.6	3.6	3.3	(2.2)	(2.0)	5.50
	17			10.1	3.3	3.4	(2.3)	(1.9)	6.18
	20			8.7	3.1	2.8	(1.9)	(1.3)	6.35
10	0	Nephrectomy 1		12.4	4.4	3.8	(2.6)	(1.9)	5.72
	3			12.6	4.3	3.9	(2.6)	(2.3)	5.70
	6			9.0	3.2	2.7	(1.9)	(1.4)	5.56
	27	Splenectomy		16.7	4.2	5.7	(3.9)	(5.3)	6.64
	31			13.2		4.3	(2.9)		6.28
	34			13.3	4.3	4.5	(3.0)	(2.6)	6.50
11	0	Splenectomy		9.8	3.4	2.8	(1.9)	(1.8)	5.37
	3			9.5	3.3	2.9	(2.0)	(1.5)	5.58
	7			10.3	3.6	3.4	(2.3)	(1.4)	5.78
	10	Nephrectomy 1		9.6	3.1	3.1	(2.1)	(1.9)	5.76
	14			10.0	3.3	3.2	(2.2)	(1.9)	6.00
	17			9.9	3.2	2.7	(1.9)	(2.2)	5.70
12	0	Nephrectomy 1		13.5	4.4	4.3	3.1	(2.5)	5.35
	5			14.7	5.0	4.7	3.3	(2.4)	5.24
	8			14.9	5.1	5.7	4.1	(1.6)	5.21
	12			14.9	4.9	5.8	(4.0)	(2.1)	—

|| 50 gm. of glucose intraperitoneally on days 7, 8, 9, and 10.

** Liver contained abnormally large amount of fat.

drop method leads to grossly inaccurate results whenever the concentration of lipid is abnormal. Lipid phosphorus, free cholesterol, and protein analyses were omitted if any hemolysis was detected.

Neutral fat was estimated by subtracting the cholesterol esters and phospholipid fatty acids from the total fatty acids, all three being expressed as milliequivalents of fatty acid per liter of serum or per kilo of dry liver. In those experiments in which the concentration of cholesterol esters in serum was not directly determined, it was assumed that, of the total cholesterol 68 per cent in the dog and 74 per cent in the monkey were esterified. In the liver it was assumed that 20 per cent of the total cholesterol was esterified. These fractions represent the averages of all analyses in which the esterified cholesterol was directly determined, and the justification for their use depends upon the approximate constancy of the ratio of esterified to total cholesterol (11). It was assumed in both serum and in liver that four-fifths of the phospholipid was present as cephalin or lecithin of conventional composition (each phosphorus combined with two equivalents of fatty acid) and that one-fifth was present as sphingomyelin (each phosphorus combined with one equivalent of fatty acid), in order to convert millimoles of lipid phosphorus to milliequivalents of fatty acid. The conversion factor, on the basis of this assumption, is 1.8. These proportions, derived from various analyses in the literature (12-16), are found in other species and are only approximate, but it may be shown that the proportion of sphingomyelin may vary considerably with but little effect on the free fat calculated in this manner (11). The formula used for neutral fat of both serum and liver is therefore:

$$\text{Neutral fat} = \text{total fatty acids} - \text{cholesterol esters} - \text{lipid P} \times 1.8,$$

all quantities being expressed in millimolar terms. The molecular weight of cholesterol is taken as 386.

RESULTS

In Table I are presented the effects in the dog of unilateral and of bilateral nephrectomy, and of splenectomy. Total fatty acids, lipid phosphorus, and cholesterol of serum rose in all seven experiments with bilateral nephrectomy. Cholesterol esters and free cholesterol both rose to about the same extent. The rise in the neutral fat was somewhat more irregular, but in every instance it rose above the initial level. In some experiments a lipid increase was evident within 48 hours, while in others it was delayed until 72 or 96 hours. There was only rough parallelism between the time of appearance and the extent of increase of the different elements. The intraperitoneal injection of glucose did not inhibit or otherwise affect this rise (Experiments 6, 7).

On the other hand, no such consistent increase took place following unilateral nephrectomy. In two of the experiments (6 and 8) there was a rise of all lipid substances 2 or 3 days after unilateral nephrectomy, while in a third (Experiment 12) the cholesterol alone rose. All elements but cholesterol tended to return toward normal within a week. In the remaining seven experiments unilateral nephrectomy was without effect on the serum lipids, either after 3

to 4 days (Experiments 2, 5, 7, 9, 10, 11), or after 7 to 10 days (Experiments 3, 5, 7, 9, 10, 11). In one experiment (9) splenectomy was followed by a transient increase in some of the lipid constituents of serum, in one (10) it was followed by a drop, and in a third (11) no change at all took place.

In Table II are presented the results of unilateral and of bilateral nephrectomy in two *rhesus* monkeys. There was no change in the serum lipids after unilateral nephrectomy. The second nephrectomy was, however, followed by a rapid rise in the lipid constituents of serum. This increase was maximal

TABLE II
Effects of Unilateral and of Bilateral Nephrectomy on the Serum Lipids and Protein of the Monkey

Monkey No.	Days after first operation	Procedure*	Blood non-protein nitrogen	Serum					
				Total fatty acids	Lipid phosphorus	Cholesterol		Neutral† fat	Total protein
						Total	Esterified†		
			mg. per cent	mm per liter	mm per liter	mm per liter	mm per liter	mm per liter	gm. per cent
1	0	Nephrectomy 1		10.4	3.1	2.8	(2.1)	(2.7)	6.75
	7	Nephrectomy 2	33	8.8	2.4	3.4	(2.5)	(2.0)	7.30
	9		162	17.3	3.9	3.8	(2.8)	(7.5)	7.14
	11		282	20.1	4.2	5.6	(4.2)	(8.3)	7.22
	12			18.6	3.6	4.6	3.4	(8.7)	6.84
2	0	Nephrectomy 1		8.1	2.9	2.9	(2.1)	(0.8)	5.98
	7	Nephrectomy 2	50	14.4	3.8	2.7	(2.0)	(10.3)	6.48
	9		222	79.4	9.9	8.0	(5.9)	(55.7)	7.47
	11		354	49.7	6.0	8.1	(6.0)	(32.9)	7.07

* After the blood samples of the same date.

† Calculated values in parentheses.

within 48 hours, and then subsided a little. In contrast to the experiments with dogs, the free fat of serum increased to a greater extent than did the cholesterol and phospholipids.

In Table III are presented the results of bilateral ureteral ligation in the dog. A rise in all lipid constituents occurred in four (15, 17, 18, 19) of the five experiments with complete ligation. The single exception (Experiment 16) was followed for 72 hours without change in the serum lipids save for a slight rise in the free fat. Experiment 20 is peculiar, in that the blood non-protein nitrogen first rose for 3 days, then gradually fell. The animal survived, presumably indicating that on the 3rd or 4th day the ligature slipped somewhat. Serum lipids rose only slightly in absolute magnitude during the first 3 days, but rose markedly relative to the concentration of protein in serum. During the period

of decline of the blood non-protein nitrogen the serum lipids again fell, relative to the concentration of protein in the serum.

TABLE III
Effects of Bilateral Ureteral Ligation on the Serum Lipids and Protein of the Dog

Dog No.	Days after bilateral ligation	Blood* non-protein nitrogen	Serum					
			Total fatty acids	Lipid phosphorus	Cholesterol		Neutral† fat	Total protein
					Total	Esterified‡		
		mg. per cent	mm per liter	mm per liter	mm per liter	mm per liter	mm per liter	gm. per cent
15§	0	36	8.9	3.3	3.2	(2.1)	(0.8)	5.04
	2	124	9.9	3.4	2.9	(2.0)	(1.9)	
	4	220	17.1	4.8	5.1	(3.5)	(5.0)	
	5	273	18.4	5.7	6.2	(4.2)	(3.9)	7.18
16§	0	72 _a	9.0	3.7	3.5	(2.4)	(-0.1)	5.82
	1		10.6	3.7	3.4	(2.3)	(1.5)	5.42
	3	500 _a	10.5	3.7	3.5	(2.4)	(1.3)	
17	0	29	9.7		3.0	(2.1)		6.63
	2	186	16.0		5.1	(3.5)		6.26
18	0	24	9.1		2.7	(1.9)		6.42
	2	130	11.6		4.0	(2.7)		6.37
19	-8		11.7		4.0	(2.7)		6.73
	-2		10.1	3.2	2.9	(2.0)	(2.2)	5.84
	0	34						
	2	190	14.3	4.9	5.0	(3.4)	(1.8)	5.98
	3	215	16.9	5.2	5.5	(3.7)	(3.6)	5.99
20	-3		10.5	4.0	4.1	(2.8)	(0.5)	
	0	33 _a	9.9	3.5	3.5	(2.4)	(1.0)	6.08
	1	95 _a	10.2	3.8	3.8	(2.6)	(0.8)	5.75
	2	186 _a	9.7	3.7	3.7	(2.5)	(0.5)	
	3	340 _a	12.4	3.8	4.0	(2.7)	(2.9)	4.56
	4	290 _a	12.8	3.9	4.1	(2.8)	(2.8)	5.12
	5	240 _a	13.9	4.3	4.5	(3.1)	(2.9)	5.89
	8	85 _a	12.1	3.6	4.1	(2.8)	(2.8)	6.22

* Subscript "s" means serum non-protein nitrogen.

† Calculated values in parentheses.

§ Low potassium diet.

Serum protein concentration changed at times following bilateral nephrectomy or ureteral ligation (Tables I, II, III), probably mainly as an expression of changes in plasma hydration (17). In Figs. 1, 2, and 3 the extent of these changes is compared graphically with the simultaneous change of each of the lipids. For comparative purposes the preoperative ratios are all taken as

unity. As a rule the ratio of each of the lipids to serum protein increased above unity following operation. This means that the increase in serum lipids after these bilateral operations is not merely an expression of a change in plasma hydration, although the latter may occasionally exaggerate the rise.

In Table IV are presented the results of four experiments with fasting and withdrawal of water for periods comparable to those during which the anuric animals of Tables I, II, and III survived. A fifth experiment in which water and glucose only were given is included. No change beyond the limits of normal variation in the serum concentration of any of the lipids took place.

In Table V are presented the results obtained by analyzing the biopsy specimens of the liver before and after bilateral nephrectomy, and before and after a period of fasting and dehydration. The control values are quite similar to those obtained in normal dog livers by Rubin, Present, and Ralli (18). Two of the nephrectomized animals received glucose injections twice daily, in order to exclude the effect of simple fasting. In this table the results are expressed in terms of 1000 gm. of dry tissue. The results are therefore independent of the degree of hydration of the tissue, but are closely dependent on any change in the other solids of the tissue. For example, as the glycogen content of the liver declines with fasting, the concentration of lipids and of all other substances *per 1000 gm. of total dry solids* must necessarily increase, even without any change in the actual total lipid content of the liver. The extent of this increase, which is merely an expression of the loss of glycogen, will be proportional to the ratio:

$$\frac{1000}{1000 - (\text{loss of glycogen from 1000 gm. of dry tissue})}$$

Unfortunately no glycogen determinations were made. It is, however, possible to set an approximate upper limit to the value of this ratio. Let us assume that, 24 hours after the last meal of chow ration, the liver of a dog will have no more than 80 gm. of glycogen per 1000 gm. of wet tissue, equivalent to 270 gm. per 1000 gm. of dry tissue. Also let us assume that after 4 days of fasting this will have been reduced to 20 gm. of glycogen per 1000 gm. of wet, or 70 gm. per 1000 gm. of dry, tissue (19). The ratio then becomes:

$$\frac{1000}{1000 - 200} = 1.25$$

A rise of as much as 25 per cent, but no more, might therefore be due to glycogen loss alone. Table V indicates that the lipid phosphorus of liver rose 51 and 73 per cent, respectively, after simple bilateral nephrectomy experiments (4 and 5). Obviously this greatly exceeds any increase due to glycogen loss alone. The increases of total cholesterol, 16 and 30 per cent respectively, were much smaller and may or may not have been significant. The small fraction of the cholesterol which was esterified did not change definitely. Fatty acids in-

creased 59 per cent in Experiment 4 but only 11 per cent in Experiment 5. Neutral fat rose slightly in one experiment and became markedly negative in the other. Such negative values are obviously impossible, and must be due to incorrect assumptions in the mode of calculation; their precise meaning

FIG. 1

FIGS. 1 to 3. These three figures compare the changes in the total fatty acids, in the lipid phosphorus, and in the cholesterol with the associated changes in the proteins of serum, following bilateral nephrectomy (crosses) and bilateral ureteral ligation (circles) in the dog. If the lipids and the proteins were changed proportionally, the ratios would be unaffected. The initial ratios are all arbitrarily taken as unity. Since the ratios usually rise in all three figures, the lipids evidently tend to increase considerably more than do the proteins of serum. The ratios of neutral fat to protein increase even more markedly. The two experiments with monkeys are not shown, as in them it is obvious that all the lipids increase much more than do the serum proteins.

will be discussed below. Certainly, however, they indicate that neutral fat did not increase.

The results in the experiments with bilateral nephrectomy in which the daily intraperitoneal administration of glucose minimized the effects due to fasting and glycogen depletion (Experiments 6 and 7A) are qualitatively similar but differ quantitatively. Thus, the lipid phosphorus increased, but less markedly, while cholesterol and cholesterol esters increased to a much greater degree. Total fatty acids did not change, and neutral fat was, if anything, reduced.

Fasting (Experiments 7B and 12) produced a moderate rise in the total

FIG. 2

FIG. 3

fatty acids (51 and 46 per cent, respectively), and a rise of about the same extent in the lipid phosphorus (33 and 25 per cent, respectively). These changes probably are significant. There were somewhat greater increases in the total

cholesterol (42 and 59 per cent, respectively). The pattern is similar to that following nephrectomy, save that the increase in fatty acids and cholesterol are perhaps more striking and that of the phospholipids somewhat less so.

TABLE IV
Effects of Fasting and of Water Depletion on the Serum Lipids and Protein of the Dog

Dog No.	Day	Procedure	Serum					
			Total fatty acids	Lipid phosphorus	Cholesterol		Neutral* fat	Total protein
					Total	Esterified*		
			<i>mm per liter</i>	<i>mm per liter</i>	<i>mm per liter</i>	<i>mm per liter</i>	<i>mm per liter</i>	<i>gm. per cent</i>
12†	0§ 4	Food and water stopped	14.9	4.9	5.8	(4.0)	(2.1)	
			13.8	4.7	5.4	(3.7)	(1.6)	5.47
7†	-3	Food and water stopped Normal diet again	13.0	4.3	4.5	(3.1)	(2.1)	5.35
	0		11.2	3.6	3.3	(2.3)	(2.5)	5.24
	5		13.5	4.5	5.0	(3.4)	(2.0)	5.21
	28		10.5		3.2	(2.2)		
21	0	Food and water stopped	9.9	3.5	3.5	(2.4)	(1.3)	5.44
	2		11.3		3.8	(2.6)		5.84
	4		9.7	3.6	3.8	(2.6)	(0.6)	5.60
	7	Water given, no food	11.3	3.5	3.2	(2.2)	(2.9)	5.45
	10		12.3	4.1	3.9	(2.6)	(2.4)	5.36
22	-9	Food and water stopped Normal diet again	8.5	2.9	3.2	(2.1)	(1.1)	4.54
	0		12.2	4.1	4.0	(2.7)	(2.0)	5.00
	4		10.9	3.7	3.4	(2.3)	(1.9)	5.04
	11		13.3	4.7	4.7	(3.2)	(1.6)	5.14
	18		12.6	4.4	4.2	(2.9)	(1.8)	5.34
23	0	Food stopped, 25 gm. glucose daily, water <i>ad lib.</i>	7.0	2.7	2.1	1.6	(0.5)	5.44
	4		7.8	2.8	2.3	1.8	(0.9)	5.43
	7		7.5	3.1	2.6	1.9	(0.1)	5.57

* Calculated values in parentheses.

† Numbers refer to dogs of Table I, which were used again in these experiments.

§ First nephrectomy (Table I) 7 days previously.

|| Just before first nephrectomy (Table I).

DISCUSSION

In 1925 Nekludov (1) noted a progressive rise of the serum cholesterol following bilateral nephrectomy or ureteral ligation in cats. More recently Heymann (2) has reported similar findings in dogs after bilateral nephrectomy and after poisoning with bichloride of mercury, and has recorded a rise of the

total fatty acids as well as of the cholesterol. These findings are confirmed in our experiments, which in addition demonstrate that the phospholipids also increase following bilateral nephrectomy. However, Heymann also found that the cholesterol and total fatty acids of serum rose after *unilateral* nephrectomy. This finding we have been unable to confirm. In two experiments

TABLE V
Effects of Bilateral Nephrectomy and of Fasting on the Lipid Content of the Liver of the Dog

Dog No.*	Day of nephrectomy or fasting	Procedure	Analyses of whole liver per kg. of dry tissue					
			Water	Total fatty acids	Lipid phosphorus	Cholesterol		Neutral fat
						Total	Esterified†	
			gm.	mm per kg.	mm per kg.	mm per kg.	mm per kg.	mm per kg.
4	0	Control	2400	224	125	22.3	4.9	(-6)
	3	Bilateral nephrectomy	2760	356	189	25.9	4.7	(+11)
5	0	Control	2640	266	147	24.0	4.7	(-4)
	0	Control	2710	325	161	20.4	0.0	(+35)
	3	Bilateral nephrectomy	2980	361	279	26.5	4.7	(-146)
6	0	Control	2860	298	158	22.2	1.6	(+12)
	4	Bilateral nephrectomy‡	3200	305	219	32.5	6.0	(-95)
7A	0	Control	2720	291	177	22.0	(4.4)	(-32)
	4	Bilateral nephrectomy‡	3020	306	198	31.5	(6.3)	(-56)
7B	0	Control	2610	300	153	21.4	(4.3)	(+21)
	5	Fasting; no water	2730	453	203	29.9	(6.0)	(+82)
12	0	Control	2370	251	138	19.5	(3.9)	(-1)
	4	Fasting; no water	3270	367	173	31.1	(6.2)	(+50)

* Numbers refer to the experiments of Tables I and IV.

† Calculated values for figures in parentheses; see text for discussion of negative values of neutral fat.

‡ Glucose treatment daily after nephrectomy.

some changes occurred suggestive of those reported by Heymann, but in eight others nothing of the sort was encountered. Similar elevations might occur sporadically after other operations, such as splenectomy, and could not be reproduced with any regularity. The reasons for the difference between our results and those of Heymann are at present obscure.

No clear cause for these increases in the lipid constituents of serum after surgical anuria could be found. They are not species specific, since they are present in the monkey as well as the dog. Since they occur as well after

bilateral renal ablation as after ureteral ligation, they cannot result from any absorption from damaged kidneys. They are not the result of fasting or dehydration, since dogs deprived of food and water failed to exhibit any such response, and injections of glucose failed to abolish or lessen it. They are not the result of anesthesia or of operative trauma, since unilateral nephrectomy, splenectomy, and laparotomy with biopsy of the liver produced no such response. They are not the result of manipulation of the kidney or of neighboring structures, such as the suprarenals, since the effect appeared only after removal of the second kidney. Finally, they are not solely expressions of contraction of the plasma volume, since the increase of the lipids usually much exceeds that of the serum proteins. The fact that all the lipid constituents tend to increase does suggest some alteration in the metabolism of fat. The excessive rise in the free fat in the monkey, as compared with the dog, may possibly be related to the fact that this animal is primarily a herbivore rather than a carnivore.

The consistent increase of the lipid constituents of the liver following bilateral nephrectomy indicates that the increased lipid content of serum is derived from extrahepatic sources. The non-specific pattern of the increase in the liver is not very different from that observed with lipid mobilization in various states (20), and is not that of a pathological "fatty liver." It seems to be a reflection rather than a cause of the serum lipid changes. The changes following fasting are very similar, although unaccompanied by any increase of the serum lipids. The liver changes cannot be due simply to fasting, however, since glucose injections failed to abolish them.

The negative values for neutral fat in the liver analyses of Table V require interpretation, since such figures are without physiological meaning, and must represent some incorrect assumption in the calculation. The mistake undoubtedly lies in the factor 1.8 which is used to convert millimols of P to milliequivalents of phospholipid fatty acid, in turn, and is based on the assumption that sphingomyelin constitutes one-fifth of the liver lipid. If the phospholipid of normal dog liver contains in fact a little larger than the assumed proportion of phospholipid combining with less than two fatty acid equivalents, giving a factor of 1.6 or 1.7, all the neutral fat values in the control liver analyses will be zero or small positive quantities. The conclusion seems inescapable, however, that the normal dog liver contains only small amounts of neutral fat. The increase in the negative values for neutral fat in the livers following nephrectomy, especially in Experiments 5 and 6, cannot be due simply to a small absolute error in the assumed conversion factor, but must represent a change in the factor as well. It should be noted that, at least under the conditions of these experiments, the total fatty acids and the lipid phosphorus of the liver are really for all practical purposes different measures of the same thing, *viz.*, of phospholipids. Total fatty acids include neutral fat and the

fatty acids of cholesterol esters and phospholipids, but quantitatively both cholesterol esters and free fat do not constitute more than a few per cent of the total in the normal liver and do not increase appreciably after nephrectomy (Table V). There is, however, an apparent paradox in Experiments 5 and 6 of Table V. Here the lipid phosphorus increases markedly following nephrectomy, while the total fatty acids increase very slightly. This is merely expressed in another way by the increasing negative values for neutral fat following nephrectomy in these experiments. The increase in the cholesterol esters is too slight to account for this discrepancy. Since there is probably little neutral fat originally present in the liver, it seems unlikely that there is any true decrease of neutral fat of this magnitude. It seems more probable that there has been a qualitative as well as a quantitative change in the phospholipids of the liver, and that the proportion of phospholipid combining with less than two fatty acid equivalents in the phospholipid of liver has greatly increased following nephrectomy.

In the present state of our knowledge it is difficult to relate this increase of serum lipids to that observed at some stages of renal disease. Elevation of serum lipids in glomerulonephritis characteristically appears most markedly during the earlier part of the disease, and tends to disappear as renal insufficiency develops (19). In fact the lipids of serum in terminal nephritis with nitrogen retention tend to be depressed rather than elevated (11, 19). Any relationship between the serum lipid changes associated with the nephrotic syndrome and those produced in these experiments must therefore be most indirect. Lipid elevation following surgical anuria may result either from cessation of some normal activity of the kidneys in fat metabolism, or as a secondary result of disturbance of other metabolic functions due to anuria. The experiments described here do not differentiate between these two speculative possibilities.

Recent work with radioactive phosphorus and with deuterium as indicators has shown a very active turnover of the phospholipids of the kidney in the course of normal metabolism, indeed almost as rapid a metabolism as that within the liver itself (21-26). The findings in our experiments may not at present be open to any simple explanation, but it seems quite possible that future work may prove that they are in some way connected with the rôle of the kidney in normal phospholipid metabolism.

CONCLUSIONS

1. In the dog and monkey bilateral nephrectomy or ureteral ligation results in a marked, progressive increase of total fatty acids, of free and esterified cholesterol, of phospholipid, and of free fat of serum.
2. No such changes follow unilateral nephrectomy, splenectomy, or fasting.

3. The increase after bilateral nephrectomy is not inhibited by glucose administration.

4. A marked increase of the phospholipid and a less significant elevation in cholesterol content of the liver accompanies this increase of serum lipids after bilateral nephrectomy.

BIBLIOGRAPHY

1. Nekludov, W. N., *Z. ges. exp. Med.*, 1925, **47**, 70.
2. Heymann, W., *Science*, 1942, **96**, 163.
3. Durlacher, S. H., and Darrow, D. C., *Am. J. Physiol.*, 1942, **136**, 577.
4. Hoff, H. E., Smith, P. K., and Winkler, A. W., *J. Clin. Inv.*, 1941, **20**, 607.
5. Man, E. B., and Gildea, E. F., *J. Biol. Chem.*, 1932, **99**, 43.
6. Man, E. B., and Gildea, E. F., *J. Biol. Chem.*, 1937, **122**, 77.
7. Man, E. B., and Peters, J. P., *J. Biol. Chem.*, 1933, **101**, 685.
8. Man, E. B., *J. Biol. Chem.*, 1937, **117**, 183.
9. Bogdanovitch, S. B., and Man, E. B., *Am. J. Physiol.*, 1938, **122**, 73.
10. Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry, Vol. II, Methods, Baltimore, Williams & Wilkins, 1932.
11. Peters, J. P., and Man, E., *J. Clin. Inv.*, in press.
12. Hunter, F. E., *J. Biol. Chem.*, 1942, **144**, 439.
13. Erickson, B. N., Avrin, I., Teague, D. M., and Williams, H. H., *J. Biol. Chem.*, 1940, **135**, 671.
14. Sinclair, R. G., and Dolan, M., *J. Biol. Chem.*, 1942, **142**, 659.
15. Thannhauser, S. J., Benotti, J., and Reinstein, H., *J. Biol. Chem.*, 1939, **129**, 709.
16. Thannhauser, S. J., Benotti, J., Walcott, A., and Reinstein, H., *J. Biol. Chem.*, 1939, **129**, 717.
17. Peters, J. P., Eisenman, A. J., and Bulger, H. A., *J. Clin. Inv.*, 1925, **1**, 435.
18. Rubin, S. H., Present, C. H., and Ralli, E. P., *J. Biol. Chem.*, 1937, **121**, 19.
19. Peters, J. P., and Van Slyke, D. D., Quantitative clinical chemistry, Vol. I, Baltimore, Williams & Wilkins, 1931.
20. Barrett, H. M., Best, C. H., and Ridout, J. H., *J. Physiol.*, 1938, **93**, 367.
21. Weissberger, L. H., *J. Biol. Chem.*, 1940, **132**, 219.
22. Sinclair, R. G., *J. Biol. Chem.*, 1940, **134**, 71.
23. Perlman, I., Rubin, S., and Chaikoff, I. L., *J. Biol. Chem.*, 1937, **122**, 169.
24. Cavanagh, B., and Raper, H. S., *Biochem. J.*, 1939, **33**, 17.
25. Chargoff, E., Olson, K. B., and Partington, P. F., *J. Biol. Chem.*, 1940, **134**, 505.
26. Chaikoff, I. L., *Physiol. Rev.*, 1942, **22**, 291.