TABLE II. Heterologous Antibody Response of Lambs to Multiple Inoculations of PR8 Influenza Virus.

Viral test antigen	HI titer of sera			
	Pre-inoculation	1st Post inoculation	2nd Post inoculation	3rd Post-inoculation
A/Sw/Wis	<8	<8	<8	64
"	<8	<8	96	64
"	<8	<8	<8	12

The PR8 titer rose from less than 8 to 64 after the first inoculation. After the second inoculation, the A/Swine/Wis titer rose from less than 8 to 512 and, as a result of this heterologous antigenic stimulation, the PR8 titer rose from 64 to 1024.

Summary and conclusions. Of 9 lambs given viable Type A influenza virus *via* the intratracheal route, only the first 2 inoculated suffered clinical illness. Their illness was not respiratory in nature. Infectious virus was not recovered from 4 animals using nasal swab materials or lung and tracheal tissues. All lambs responded immunologically to primary intratracheal inoculation of virus, and second inoculations resulted in significant rises in homologous HI antibody titer. Further inoculations of virus served to broaden the antibody response without increasing the peak homologous antibody titer. The antibody titers reached suggest the feasibility of using lambs for large-volume production of anti-influenza typing sera.

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Post-Heparin Plasma Lipoprotein Lipase Levels in Cirrhosis of the Liver.* (28235)

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Hahn(1) observed that injection of heparin into dogs showing alimentary lipemia resulted in the disappearance of plasma turbidity. This was due to release into the circulation of a substance called clearing factor(2) and subsequently shown to be a lipoprotein lipase(3).

The precise role of lipoprotein lipase in the metabolism of triglycerides is not certain although it may permit the clearing of chylomicron triglycerides from the circulation(4). Circulating lipoprotein lipase is removed by the isolated rat liver(5,6) and also by the human liver(7), while high levels of post-heparin lipoprotein lipase activity have been reported in rats and humans with liver damage (8,9,10). It has therefore been inferred that the normal liver removes lipoprotein lipase from the circulation while the diseased liver fails to do so. Most of the results leading to this conclusion have been obtained by measuring lipoprotein lipase activity turbidometrically or under suboptimum conditions.

The present work describes an improved method of estimation of lipoprotein lipase activity measuring initial velocity of the reaction permitting zero order kinetics. Results are given for post-heparin plasma lipoprotein lipase activity in control and cirrhotic subjects.

Materials and methods. The 31 patients comprised 10 with postnecrotic cirrhosis, 10 with primary biliary cirrhosis, 9 with juvenile

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cirrhosis(11) and 2 with alcoholic cirrhosis. The control subjects were laboratory staff. The subjects received a normal diet and had fasted overnight and were resting. Ten ml of blood were withdrawn from the antecubital vein using a chilled syringe and then heparin (10 units per kg body weight) was rapidly given intravenously. A second blood sample was withdrawn 10 minutes later. It has been observed in 4 normal and 4 patients suffering from cirrhosis of the liver that post-heparin lipoprotein lipase activity was maximal at 10 minutes. The blood was kept in frozen test tubes containing 10 to 20 units of heparin and plasma separated by centrifuging at 0°C for 30 minutes at 3000 rpm. The separated plasma was used immediately or frozen for subsequent assays.

The coconut oil emulsion used as substrate consisted of coconut oil B.P. 30%, sucrose 12.5%, glycerol monostearate 1.5%, Tween 60 2% and water to 100 ml. The constituents were first emulsified in a mechanical stirrer at 70°C and then by ultrasound for ½ hour. This emulsion was diluted to comprise 5% coconut oil using normal saline. The particle size of emulsion was shown to be under one micron.

Preliminary kinetic studies established the following optimum conditions for measuring lipoprotein lipase activity: 0.3 ml of coconut oil emulsion (containing 15 mg of triglycerides), 0.1 ml of 0.5 M calcium chloride as the fatty acid acceptor, 0.5 ml of 0.1 M Tris buffer pH 8.9 and 0.1 ml of plasma in a total volume of 1 ml were placed in a 15 ml glass stoppered test tube. This was incubated in a shaking water bath at 37°C. Duplicate test tubes were removed at 10, 20 and 30 minutes. The reaction was stopped by adding 5 ml of free fatty acid extraction mixture and the free fatty acids (FFA) liberated were estimated by Dole's(12) method. The FFA concentration less the blank values were plotted against time and slope of line used to calculate the reaction velocity in μeq FFA liberated per min/ml of plasma.

Results. The mean post-heparin plasma lipoprotein lipase activity of 20 normal fasting subjects was 0.46 (S.D. ± 0.13) μeq FFA liberated/min/ml of plasma. The fasting levels were reduced in 31 patients with cirrhosis of the liver (Table I). No consistent correlation was observed between lipoprotein lipase activity and clinical features and body habitus or serum bilirubin, alkaline phosphatase and cholesterol levels.

TABLE I. Post-Heparin Plasma Lipoprotein Lipase Activity (μeq FFA/min/ml of Plasma).

Subjects	No.	Mean and S.D.	Range
Controls	20	.460 ±.130	.310-.730
Primary biliary cirrhosis	10	*.066 ±.038	.017-.140
"Juvenile cirrhosis"	9	*.022 ±.020	.00 -.73
Postnecrotic cirrhosis	10	*.095 ±.054	.04 -.164
Alcoholic cirrhosis	2	.115 —	.041-.190

* Significant difference from controls ($p < .001$).

Discussion. Post-heparin plasma lipoprotein lipase activity has been measured in normal subjects and patients suffering from cirrhosis of the liver using a new system functioning at optimum conditions. Sonication by ultrasound was used to give a fine, homogenous and stable triglyceride emulsion. The use of calcium chloride obviated the difficulty of extraction of fatty acids met with in systems using albumin as the fatty acid acceptor. No increased activity was observed by adding both albumin and calcium to the system. This method gives a considerably higher post-heparin lipoprotein lipase activity in normal subjects than that obtained by other methods (7,13,14,15). This is presumably because it permits measurement of initial rates, contains optimal concentrations of standardized artificial coconut oil emulsion as a substrate, and of calcium chloride as fatty acid acceptor, and works at optimal pH and temperature. Frederickson and Ono(16) recently reported comparable but lower activity in post-heparin plasma using adequate substrate and albumin as a fatty acid acceptor.

The present study, in contrast to the observations of others(8,9,10), has shown low lipoprotein lipase activity in patients with cirrhosis of the liver when lipolysis has been used as the index of enzyme activity. Previous studies, however, have been based on the decrease in turbidity of the system which is not always a valid procedure(17).

No information is available as to the reason for the low lipoprotein lipase activity in cir-

rhotic patients. Fat deprivation for a long period is known to lower post-heparin lipoprotein lipase response(16) and the steatorrhoea demonstrated in some, but not all, of our patients with cirrhosis of the liver might contribute to the findings.

Plasma free fatty acids fail to rise after administration of heparin in fasting patients with cirrhosis(18). This might be at least partly explained by the low lipoprotein lipase activity.

Summary. An improved method is described for estimating post-heparin plasma lipoprotein lipase activity permitting initial velocity measurement with zero order kinetics. Plasma values after heparin in 20 normal subjects are considerably higher than previously recorded. Values in 31 cirrhotic patients are significantly lower than normal.

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Effect of Carcinogenic Polynuclear Hydrocarbons on Corticosterone and Ascorbic Acid Content of Adrenal Glands in Rats.* (28236)

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The induction of massive necrosis and hemorrhage in the adrenal cortex by 7,12-dimethylbenz(a)anthracene (DMBA) reported earlier by Huggins and Morii(1) has since been confirmed(2,3). The adrenolytic effect of DMBA was found to involve selectively the inner zones of the adrenal cortex. Interestingly, this profound effect of DMBA is not a property common to all carcinogenic hydrocarbons. A single large dose or repeated doses of 3-methylcholanthrene (3MC) failed to induce adrenocortical necrosis. It was demonstrated, however, that so far as induction of mammary cancer in rats was concerned, 3MC was a much less potent carcinogen than DMBA.

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In the present investigation, it was observed that concentrations of corticosterone in the adrenal gland and in plasma decreased rapidly after a single dose of either DMBA or 3MC. It was also demonstrated that whereas ascorbic acid synthesis was greatly depleted in the adrenal glands of DMBA-treated rats, it was, to the contrary, stimulated in the adrenal glands of rats receiving 3MC. These observations suggest that the mechanisms by which these 2 polycyclic hydrocarbons suppress corticosterone secretion in the adrenal cortex may not be the same, since 3MC does not induce adrenocortical necrosis.

Materials and methods. The experimental animals were female Sprague-Dawley rats 50-55 days old and weighing 150-185 g. The car-