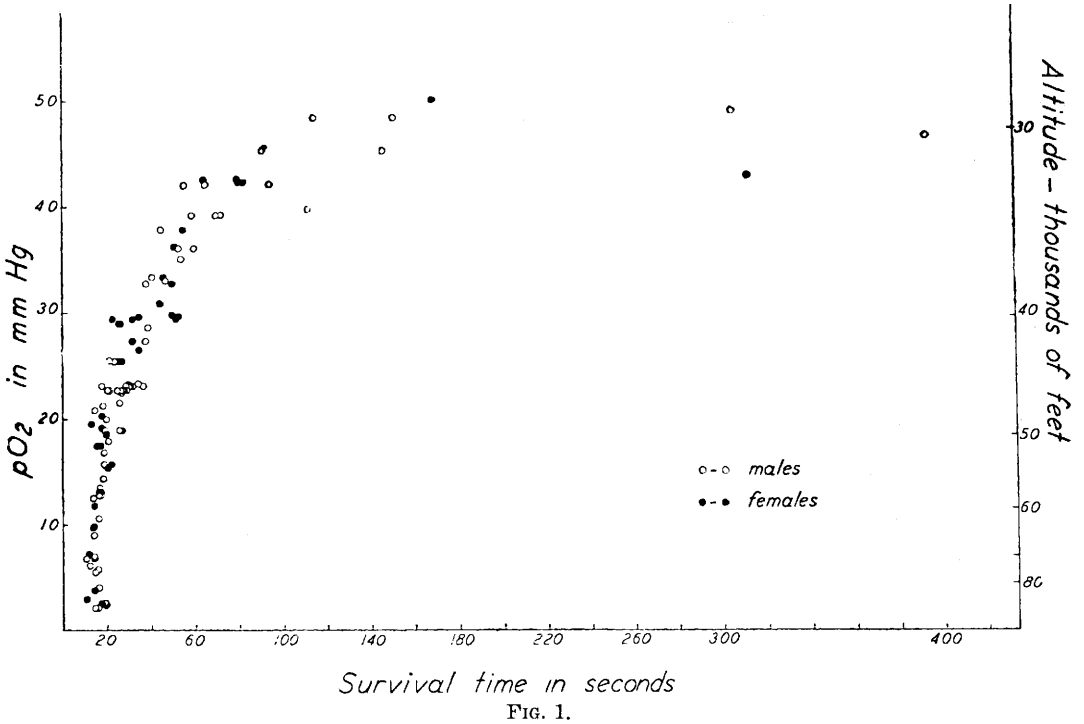

FIG. 1.

termined. A critical barometric pressure of 100 mm Hg exists below which no further reduction in survival time occurs. The pO_2 at which survival no longer is dependent on oxygen pressure is therefore about 21 mm Hg. No sex difference in survival of mice was found.

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Serum Lipids and Their Fractionation in Alloxan Diabetes in the Rabbit.* (17673)

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In 1945 Kendall *et al.*(1) showed that a transient hyperlipaemia and hypercholesterolaemia may or may not occur during the early stages of uncontrolled alloxan diabetes in the rabbit. Duff and Wilson(2) subsequently confirmed these findings, making a more careful study of the serum cholesterol over a longer period of time. So far as we are aware, however, there has been no detailed study of the other serum lipids in alloxan diabetic rabbits. The lipids of the serum are characterized by two important features: in the first place the different lipid components are known to show an interrelationship which is more constantly maintained and, therefore, of more

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2. Duff, G. L., and Wilson, D. C., unpublished observations.

significance, than the concentration of any single component(3); secondly, but of no less importance, there is a close association between the serum lipids and the serum proteins, and some sort of lipoprotein complex has been suggested to explain the lack of turbidity of normal serum(4). In any detailed analysis of the serum lipids, therefore, it is necessary not only to determine the absolute levels of the various lipid constituents, but also to examine the relationship of these constituents to the serum proteins. Absolute lipid levels can be determined with a reasonable degree of accuracy, but the study of the relationship of the lipids to the serum proteins is fraught with difficulty. The recent publication of Forbes *et al.*(5), however, offered a reproducible method which was considered likely to reflect the state of binding of the lipids in one or more of the serum lipoproteins. The normal range of the serum lipids was first studied in order to establish a base line with which the values obtained from the diabetic sera might be compared. This was of particular importance in regard to the values obtained by the method of Forbes, since serum total cholesterol is the only lipid constituent that has been studied by this method.

Experimental procedure. Young adult male albino rabbits were used. As a preliminary step forty-two normal animals were studied by the methods described below. For the experiment proper a number of these rabbits were made diabetic by the intravenous administration of Alloxan (Eastman Kodak) in 5% aqueous solution. A single dose of 200 mg per kg of body weight was used. All the animals received the same diet—an unlimited amount of Purina Laboratory Chow and water. Fourteen rabbits became diabetic and maintained a blood sugar of about 300 mg % or more; two rabbits showed only a transitory hyperglycemia and were termed "alloxan recovered". Serum lipid determinations on these sixteen animals were made one or two weeks after the injection of alloxan and subsequently at intervals of about two weeks.

Blood was drawn in the non-fasting state from the central artery of the ear and the serum separated. The presence or absence of visible lipaemia was noted. Half of the serum was used immediately for the determination of the lipid constituents. Total fatty acids were determined by the method of Stoddard and Drury as modified by Man and Gildea(6); lipid phosphorus by the method of Youngburg as modified by Hawk, Oser and Summerson(7); free and total cholesterol by the method of Schoenheimer and Sperry as modified by Sperry(8). Neutral fat was calculated as described by Peters and Man(9). The other half of the serum was rapidly frozen and then dried *in vacuo* and extracted overnight with chloroform in the cold according to the method of Forbes(5). The chloroform was then evaporated *in vacuo* at a temperature below 40°C and determinations of total fatty acids, lipid phosphorus and free and total cholesterol were made on the residue as above. The values obtained in this latter way will be referred to as the "readily extractable lipid fractions", representing, according to Forbes(5), the lipid fractions either not bound or only loosely bound to protein. Blood sugar content was determined by a modification of Folin's micro method(10) at the time of each lipid analysis.

Results. Table I summarizes the results of this study. From a consideration of the

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10. *Notes on Operation of Evelyn Photoelectric Colorimeter,* Rubicon Co., Philadelphia, Pa.

TABLE I.
Serum Lipids and Their Fractionation in Alloxan Diabetic Rabbits.

Date		Wt (kg)	Blood sugar (mg%)	Fatty acids of neutral fat (meq.) Abs.	Fatty acids of neutral fat (meq.) R.E.	Lipid phosphorus (mg%) Abs.	Lipid phosphorus (mg%) R.E.	Cholesterol (mg%) Abs. Total	Abs. Free	Abs. Ester	R.E. Total	R.E. Free	R.E. Ester	Visible Lipaemia
Rabbit A 66 Diabetic—representative of Group I (5 animals).														
Nov.	29/48	2.86	125	7.6	7.3	3.1	0.2	38	13	25	11	6	5	0
Dec.	2	3.18	Alloxan administered (200 mg/kg)											
”	15	3.35	400	10.0	5.8	3.9	0.2	30	20	10	16	3	13	0
”	28	3.39	440	7.4	5.3	3.6	0.3	46	25	21	34	8	26	0
Rabbit A 61 Diabetic—representative of Group II (8 animals).														
Nov.	24/48	2.72	100	5.1	5.7	2.6	0.3	50	15	35	14	3	11	0
”	30	2.89	Alloxan administered (200 mg/kg)											
Dec.	15	2.53	330	88.6	65.7	20.7	14.0	270	197	73	208	162	46	+
”	28	2.49	440	16.0	7.7	5.3	0.8	70	30	40	26	7	19	±
Jan.	10/49	2.63	360	9.5	3.3	3.3	0.5	55	13	42	30	13	17	0
Rabbit A 59 Diabetic—representative of Group III (2 animals).														
Nov.	22/48	2.79	115	6.0	3.7	4.0	0.2	22	15	7	10	3	7	0
”	30	2.86	Alloxan administered (200 mg/kg)											
Dec.	12	2.83	510	226.2	202.0	46.0	36.4	615	485	131	470	395	75	+
”	28	2.83	485	49.0	35.6	12.9	6.2	120	83	37	82	50	32	++
Jan.	10/49	2.83	435	145.9	137.6	30.0	18.8	330	265	65	316	257	59	++
”	20	2.70	520	47.0	23.0	12.5	2.7	136	106	30	110	48	62	++
Feb.	1	2.98	400	176.8	171.0	34.4	19.1	282	113	169	244	130	114	+
Rabbit A 4—representative of “Alloxan Recovered” Group (2 animals).														
Oct.	31/48	3.17	95	8.2	7.4	2.8	0.2	40	13	27	12	11	1	0
Nov.	13	3.29	Alloxan administered (200 mg/kg)											
Dec.	1	3.78	190	6.9	2.0	2.8	0.4	18	11	9	16	5	11	0
”	12	4.09	120	2.9	3.2	2.7	0.3	38	17	21	16	3	13	0

Note: Abs. = Absolute levels. R.E. = “Readily extractable.”

TABLE II.
Normal Range of Serum Lipids as Determind on 42 Normal Rabbits.

	Fatty acids of neutral fat (meq.)		Lipid phosphorus (mg%)		Cholesterol (mg%)			
					Total		Free	
	Mean	S.Dev.	Mean	S.Dev.	Mean	S.Dev.	Mean	S.Dev.
Absolute lipid levels	7.3	3.1	4.4	1.7	46	17.5	19	9.5
"Readily extractable" lipid levels	6.2	2.7	0.3	0.01	21	12.2	8	6.8

absolute lipid levels alone the diabetic animals were found to fall into three groups and for each group the values for one representative animal are given. The determinations in one of the "alloxan recovered" animals are also shown. It will be seen that in the first group, consisting of five animals, there was no conspicuous change in serum lipids; the second group, which consisted of seven animals, showed a brief and temporary rise in serum lipids with a return to normal within three to six weeks; the third group, composed of two animals, showed an elevation in serum lipids that persisted with fluctuations for an indefinite length of time. The two "alloxan recovered" animals behaved like the diabetic rabbits of the first group in that they showed no lipaemia. It was noted that the diabetic animals of group I as well as the "alloxan recovered" animals all gained weight after the administration of alloxan while the diabetic rabbits of group II and III showed a definite loss of weight after the inception of the diabetic state. The rabbits continued to eat well after becoming diabetic and, indeed, the diabetic animals of group III ate ravenously. The diabetic rabbits of group III showed a greater elevation of blood sugar than did those of the other two groups.

Closer study of the absolute lipid levels revealed that when any one of the lipid constituents was elevated, all the others were also elevated, though not to the same degree, and there usually was a visible lipaemia. Relative to normal proportions the fatty acids of neutral fat were most elevated, and the lipid phosphorus least increased; in proportion to the other two lipid constituents, the increase in total cholesterol was intermediate in position. The elevation of total cholesterol was due much more to an increase in free cholesterol than to an increase in ester cholesterol and there was a correspondingly marked change in the ratio between the two. This change in ratio occurred even when there was no rise in the total cholesterol or in the other lipid constituents.

When the "readily extractable" lipid fractions were compared with those obtained by the ordinary methods, several interesting points became evident. Table II shows this comparison in the normal rabbit. It will be seen that in the normal animals the most striking feature was that only a very small portion of the lipid phosphorus was "readily extractable". In addition, the values for total and free cholesterol were usually not more than half of those obtained by routine methods, but varied somewhat from animal to animal. The greater part, if not all, of the fatty acids of the neutral fat were "readily extractable". In the diabetic rabbits when there was a lipaemia, there were definite alterations in this normal ratio between the absolute values and the values for the "readily extractable" lipid fractions. These changes (Table I, Rabbits A61 and A59) were as follows: when the lipid phosphorus was markedly elevated, there was a marked increase in the "readily extractable" fraction of this lipid constituent; the proportion of "readily extractable" total and free cholesterol was also increased when the serum cholesterol was elevated. The proportion of "readily extractable" neutral fat did not change. When there was no lipaemia (Table I, Rabbits A66 and A4) no definite alterations in the "readily extractable" lipid fractions were noted.

Discussion. From our results it is clear that alloxan diabetes in the rabbit may or may not be accompanied by an elevation in serum lipids. Whenever an elevation oc-

curred, it was usually transitory, disappearing within six weeks after the inception of the diabetic state, although in a few cases it persisted with fluctuations for an indefinite period of time. In such hyperlipaemia, whether transitory or not, all of the lipid constituents of the serum were increased, the neutral fat showing the greatest increment, a finding which also occurs in the diabetic hyperlipaemia of man(11) and of depancreatized dogs fed raw pancreas before and after insulin deprivation(12). In the diabetic rabbits in which the serum lipids were elevated, there was always a concomitant loss of body weight and, with the subsidence of the lipaemia, a gain in weight was noted. In contrast, those animals, in which there was no lipaemia, always showed a gain in weight. All of the rabbits continued to eat well after becoming diabetic. In addition, in the animals in which the elevation of serum lipids was most marked the degree of hyperglycaemia was greatest, although there was little difference in blood sugar level between the diabetic animals that showed only a moderate hyperlipaemia and those that showed none at all. From a consideration of these findings it appears that the elevation of serum lipids was due to a mobilization of fat from the body depots to meet the excessive demand for fat combustion caused by the disturbance in the regulation of carbohydrate metabolism. Our animals were never in diabetic coma and exhibited none of the signs of dehydration so that the factor of haemoconcentration(11) could hardly have played any part in the elevation of the serum lipids. At each determination not more than 10 cc of blood were drawn and it is known that the rabbit will tolerate the repeated loss of much larger quantities of blood than this without any change in the serum lipids(13).

It is interesting that the rise in total cholesterol was due much more to an increase in free cholesterol than to an elevation of cholesterol esters. This observation has already been made in the alloxan diabetic rabbit(2) and in the depancreatized dog fed raw pancreas before and after insulin deprivation(12). Consideration of the values obtained by the method of Forbes showed that from normal rabbit sera only a small proportion of lipid phosphorus and usually half, or less, of total and free cholesterol were "readily extractable", while all or nearly all of the neutral fat could be extracted by this method. In the diabetic animals, however, when the serum lipids were elevated, there was a marked increase in the proportion of "readily extractable" lipid phosphorus and the proportion of "readily extractable" total and free cholesterol was also increased. The neutral fat showed little change. It would appear, therefore, that in the normal rabbit the serum lipid phosphorus and cholesterol are to a large extent bound to the serum proteins; this has been suggested to be the case in normal human serum on the basis of electrophoretic studies(14). On the other hand, in the hyperlipaemia of alloxan diabetes in the rabbit the increase in lipid phosphorus and cholesterol appears to be largely in the fractions not closely bound to protein. Neutral fat did not appear to have any close association with protein, the irregularity of its behaviour being attributable to the errors inherent in its calculation.

Summary. In the early stages of alloxan diabetes in the rabbit there may or may not occur a transitory hyperlipaemia in which all of the serum lipid constituents, but especially the neutral fat, are elevated. In a few cases the hyperlipaemia may persist for an indefinite period of time. This elevation of serum lipids is apparently due to mobilization of fat from the tissue fat depots and is related to the severity of the diabetes. In the normal rabbit only a small proportion of the lipid phosphorus and the total and free cholesterol is "readily extractable"; in the hyperlipaemic serum of the alloxan diabetic rabbit a much greater proportion of these lipid constituents is "readily extractable". It is suggested that

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in the normal rabbit much of the lipid phosphorus and cholesterol of the serum is bound to the serum proteins, while in the hyperlipaemia of alloxan diabetes in the rabbit the increase of these lipids is principally in the fractions not closely associated with the serum proteins.

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Increase in Glyceride Content of Brown Fat by Treatment with Adrenocorticotropin.* (17674)

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Although the important role played by adrenocorticotropin (ACTH) and the hormones of the adrenal cortex in the metabolism of carbohydrate and protein is well established, less is known concerning their effects on the metabolism of fat. However, it is becoming apparent that ACTH may modify the distribution of visible fat in the body. Thus, it has been demonstrated previously that ACTH may cause the disappearance of almost all of the fat of the tela subcutanea(1) while increasing the fat content of the liver (2,3) and marrow of the tibia(4). During the course of autopsies on animals treated with ACTH, it was observed that the brown fat of the interscapular gland had taken on somewhat the appearance of white adipose tissue. Therefore, we have undertaken to analyze this change with microscopic techniques.

Methods. The interscapular gland was studied from 10 adult male rats given 1 mg of ACTH daily for 21 days and fed a high fat diet by stomach tube and 3 castrated rats given the same dosage but a medium carbohydrate diet. For each treated rat there was 1 control. In addition 6 hypophysectomized rats were given 3 mg of ACTH daily for 21 days by *continuous* subcutaneous injection and fed *ad libitum*. In addition, brown fat in other locations was available for study in microscopic preparations made from many animals treated with ACTH as reported in our previous publications. These areas included the brown fat in the region of the kidneys, adrenal glands, superior mediastinum and neck. Samples of the interscapular gland were fixed in Bouin's fluid, 10% neutral formalin and chilled 80% alcohol and Carnoy's fluid. Lipids were stained with Sudan red, Sudan black B, Schiff reagent, Nile blue sulphate, subjected to the Schultz reaction, and also studied for auto-fluorescence and birefringence. The Gomori(5) procedure was used for alkaline phosphatase with sodium glycerophosphate and fructose diphosphate serving as substrates. Best's carmine and the Hotchkiss(6) periodic acid-leucofuchsin techniques were utilized for the demonstration of glycogen. Staining for cytoplasmic basophilia

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