no significant variation in sugar concentration of the plasma. It would therefore appear that the sex difference in blood glucose values reported for the chicken is a manifestation of variations in cell concentration. Androgen alters the whole blood sugar level by its cell-increasing action rather than by a primary action on sugar concentration.

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Low Molecular Weight Hexosamine-Containing Compounds in Urine.*† (22405)

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Hexosamines‡ are important components of connective tissue, synovial fluid, plasma and urine, and are frequently increased in amount in connective tissue diseases such as rheumatic fever, rheumatoid arthritis and disseminated lupus erythematosus(1-3). Hexosamines in the body are, almost exclusively, constituents of high molecular weight compounds (*e.g.* mucopolysaccharides, mucoproteins). They are not known to exist in a free state in nature(4) and even dialyzable hexosamine-containing compounds have not heretofore been demonstrated in body fluids and tissues.

It has been recently established by several investigators that a large non-dialyzable hexosamine fraction exists in normal urine(3,5,6). The development of an ion exchange method for the isolation of hexosamines(7) has made it possible to study quantitatively all hexosamine compounds in urine. During the course of such a study it became evident that a portion of the total urine hexosamine was freely dialyzable(3). Data are presented here which indicate that these substances form a relatively large pool of heretofore unknown low molecular weight hexosamine-containing compounds.

Methods. Urine specimens were collected for 24 hours and either studied directly or frozen until ready for analysis. Dialysis of urine was carried out at 4°C in large volumes of distilled water (1:50). The water was changed 2 times at 24 hour intervals. The difference between the concentration of hexosamine in the bag before and after dialysis (with appropriate volume corrections) was used to calculate the per cent of dialyzable hexosamine-containing compounds in urine. After acid hydrolysis and subsequent isolation

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‡ Naturally occurring hexosamines are glucosamine and galactosamine. The method used here does not distinguish between these two amino sugars.

TABLE I. Urinary Excretion of Hexosamine in a Variety of Clinical Conditions.*

Diagnosis	No. observations	Hexosamine		
		Total, mg/24 hr	Dialyzable fraction	
			mg/24 hr	% of total
Myxedema—Case 1	5	52 ± 5	16 ± 3	33 ± 6
Idem 2	3	53 ± 3	20 ± 4	37 ± 5
Cholecystectomy, pre-op.	1	129	54	42
" 2 days post-op.	1	303	137	45
" 13 " "	1	106	45	42
Pregnancy, 3rd trimester	13	159 ±24	67 ±13	42 ± 5
Nephrosis	1	508	89	17
Normal adults	8	79 ± 9	37 ± 4	47 ± 5

* Stand. dev. $= \pm\sqrt{\frac{\Sigma x^2}{(n-1)}}$.

on Dowex-50, hexosamines were determined using a modification of the method of Elson and Morgan(7). Further studies on dialyzable hexosamine compounds were carried out on a lyophilized dialyzate of 20 liters of pooled normal male urine.§

Results. The relatively large amounts of low molecular weight hexosamine-containing compounds excreted in a variety of clinical conditions are shown in Table I. Normal adults excreted 79 mg of hexosamine per 24 hours, of which 47% was dialyzable. Myxedema, a condition known to be associated with low excretion of total hexosamine(3,8), also demonstrated a low excretion of dialyzable hexosamine. On the other hand, conditions known to be associated with increased excretion of total hexosamine (surgery(3), pregnancy(9)) demonstrated increases in the dialyzable fraction. This was particularly striking on the second post-operative day in the patient who had a cholecystectomy for obstructive jaundice, and a series of pregnancies studied in the third trimester. In both instances the percentage of dialyzable hexosamine excreted remained normal. In a case of juvenile nephrosis the total hexosamine excretion was markedly increased. This was due in large part to the presence of non-dialyzable hexosamine-containing plasma proteins.

Discussion. Hexosamines are present abundantly in many body tissues and fluids (4,7) in the form of mucoproteins and mucopolysaccharides. Little is known concerning the intermediary metabolism of these high molecular weight compounds. Mucopolysaccharides, when treated enzymatically (hyaluronidase, glucuronidase) can be broken down *in vitro* to low molecular weight disaccharides and oligosaccharides. The dialyzable hexosamine-containing compounds demonstrated here may represent fragments of these high molecular weight compounds.

Since these low molecular weight hexosamine-containing compounds represent only about 0.002% of the total urinary solids, their identification will depend primarily on chemical isolation methods. Preliminary gradient elution studies on cellulose columns have demonstrated at least three distinct low molecular weight hexosamine fractions(10).

Summary. Relatively large amounts of low molecular weight hexosamine-containing compounds were shown to be present in urine. The excretion rate varied from 16 mg/24 hours (myxedema) to 137 mg/24 hours (surgical stress) as compared with a normal mean excretion rate of 37 mg/24 hours.

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Unesterified Fatty Acids and Lipid Transport in Dogs.* (22406)

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This study was undertaken to investigate the relative importance of two lipid transport mechanisms, lipoproteins and unesterified fatty acids.

Materials and methods. Alimentary lipemia was produced in male, mongrel dogs by feeding 40 ml of cod liver oil. Blood samples were oxalated, promptly refrigerated, and centrifuged in the cold. Unesterified fatty acids were determined by the method of Davis(1) as modified by Grossman(2). Esterified fatty acids were estimated by the procedure of Stern and Shapiro(3). The sugar content of the plasma was determined by the method of Somogyi(4) with Nelson's colorimetric adaptation(5). All analyses were in duplicate.

Results. During absorption, most fats are transported in the blood stream as neutral lipids. It seemed important to know whether under these conditions the dog also uses the other common transport mechanism, unesterified fatty acids. Fig. 1 presents one of the experiments in which esterified and unesterified fatty acids were determined simultaneously during fat absorption. Both esterified and unesterified fatty acids increase in concentration, the latter usually somewhat more slowly. The fasting dog (Fig. 1) fails to show these changes.

It has been shown(6-8) that the injection of heparin raises the level of unesterified fatty acids in the blood. We undertook to learn whether protamine reverses this action. Fig. 2 shows the rise of the unesterified fatty-acid level following the injection of heparin into lipemic dogs, the appearance of clearing factor,† and the lowering of the esterified fatty-acid level of the plasma.‡ All 3 effects can be detected in the blood from the femoral artery and vein, right side of the heart, hepatic, portal, renal, and jugular veins. The administration of protamine to these animals, subsequent to heparin, has the opposite effect, a decrease in the unesterified and a rise in the esterified fatty-acid levels and the disappearance of clearing activity from the plasma. It can also be seen that the complete disappearance of clearing activity from the plasma does not occur immediately after the injection of protamine. A definite, but decreased, clearing activity persists for 2 to 3 minutes. This suggests that the *in vivo* inactivation of clearing factor by protamine may be more complicated than a simple binding of heparin. These experiments imply that the action of heparin and of protamine involves both lipid transport mechanisms, esterified and unesterified fatty acids.

Since unesterified fatty acid transport is probably of secondary importance during fat absorption when lipids of exogenous origin travel in the blood stream, it seemed important to investigate conditions under which endogenous lipids are transported in the blood.

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† These effects can also be demonstrated in nonlipemic dogs and in dogs fasting for 2 to 3 days.

‡ "Clearing activity of the plasma," as shown in the Figure, is the clearing in optical density units of 1 ml of a cottonseed-oil emulsion by 0.5 ml of plasma after 5 hours' incubation at room temperature.