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The Transfer of [³H]Stearic Acid from Chylomicra to Milk Fat in the Goat

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It has been thought probable for a long time that the ruminant udder uses plasma lipid as a source of milk fat in addition to synthesizing some from acetate. An arteriovenous difference of neutral fat during lactation has been reported by Lintzel (1934) in the goat and by Graham, Jones & Kay (1936) in the cow. Work with isotopes also has suggested that the ruminant udder can incorporate blood triglycerides into milk fat. Glascock, Duncombe & Reinius (1956) showed that dietary [³H]stearic acid was transferred to milk fat, as did Riis, Luick & Kleiber (1960) for colloidal plasma lipid. There is, therefore, evidence that the mammary glands take up long-chain fatty acids from the blood triglycerides. It is not, however, decisive, because of the metabolism of other organs in the whole animal and the failure to demonstrate (Glascock, McWeeny & Smith, 1957) a conventional 'precursor-product' relationship (Zilversmit, Entenman & Fishler, 1943). The attempts of Glascock (1958) to obtain more quantitative information by the intravenous injection of

labelled fat emulsions were unsatisfactory because they were rapidly removed from the circulation. Further, Glascock *et al.* (1957) reported that a goat died after an intravenous injection of gelatin-stabilized fat emulsion, and in a cow 'acute symptoms' developed after the injection of only 300 mg. of emulsion stabilized with lecithin and glycerol monostearate. Similarly, T. Gillman & W. M. F. Leat (personal communication) observed that a commercial preparation (Infonutrol) used successfully in man can cause collapse and sometimes death in adult sheep. In isolated perfused goat mammary glands we have observed a fall in blood flow and haemolysis with neutral emulsions of triglycerides and long-chain fatty acids, stabilized with bilirubin or lecithin in albumin-Krebs solution (D. C. Hardwick & J. L. Linzell, unpublished work). For this reason we do not normally include fat in our substrate mixture, although in some experiments a fall in milk fat concentration after some hours of perfusion suggests that the tissue may need it.

The aim of the present experiments was first to test the use of goat chyle (intestinal lymph) rich in chylomicra as a source of fat for the perfused goat's

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udder, and secondly by infusing chylomicra into conscious lactating goats and into the perfusion medium of isolated mammary glands, to seek unequivocal evidence for the ability of lactating mammary glands to incorporate plasma fat directly into milk fat.

METHODS

Preparation of chylomicra. The main lymph duct draining the intestines was cannulated in four young castrated male goats by the technique described by Lascelles & Morris (1961) for the sheep. The lymph cannula was protected where it came through the skin by the flexible holder used by Linzell (1960). After the operation, while chyle was flowing, [^3H]stearic acid (nominally $[9,10-^3\text{H}_2]\text{stearic acid}$, from The Radiochemical Centre, Amersham, Bucks.) was dissolved in olive oil and milk and either given by mouth or injected via a cannula in the duodenum, and the lymph containing radioactive chylomicra was collected. In three animals (3 months old) only one collection of radioactive lymph was made. In the fourth animal (5 months old) lymph flowed continuously (about 1 l./day) until the animal was killed after 10 days. In this goat five doses ranging from 50 to 500 μC of [^3H]stearic acid were injected over a period of 4 days through a cannula into the duodenum, and the radioactive chyle was collected during the ensuing few hours after each injection. The animal was fed on a standard cereal ration (containing about 4% of fat) and hay. Although it had been drinking milk before the operation it took very little afterwards and drank 0.9% NaCl in preference to water.

The chyle was collected in sterile polythene bottles containing penicillin and neomycin and allowed to clot. The fibrin was filtered off through glass wool, leaving a milky fluid, containing about 2% of fat, which was stored at 4° until used. Some chyle stored at -10° for 3½ months and used for Expt. 143 was unsatisfactory (see the Results section). Some of the chylomicra were separated by ultracentrifugation at 65000g for 30 min. under 0.15 M-NaCl (Bragdon, 1958) and resuspended in 0.15 M-NaCl.

Whole-animal experiments. The chyle was injected intravenously into 5 goats (4 lactating, 1 dry) to determine the half-life of the labelled chylomicra in the circulation, and their distribution in the tissues. The animals remained undisturbed, often eating and ruminating during the experiments. Two animals (Jill and Bessie) were in the

second half of lactation, giving respectively 70 and 130 ml. of milk/hr., which was 45 and 66% of the peak daily milk yield achieved earlier in the lactation. They were milked out after the intravenous injection of oxytocin at the morning milking and thereafter at hourly intervals (again after the injection of oxytocin). At the second hour 50 ml. of chyle containing 2.09% of fat (13.5 μC of ^3H) was injected intravenously in about 20 sec., and blood samples were collected at 1, 2, 4 and 8 min. from an exteriorized carotid artery and at 15, 30, 60, 120 and 240 min. from the jugular vein.

The two other lactating goats (Expts. 143 and 144 in Table 2) that were given similar quantities of chyle intravenously were killed at 21 and 16 min. after injection by shooting through the head. One mammary gland from each animal and other tissues were taken within 1 min. and were frozen at once in liquid air. The other mammary gland was washed through with ice-cold 0.9% NaCl in a bucket of cold 0.9% NaCl. It was then perfused in isolation with homologous blood. In Expt. 143 the animal was under spinal anaesthesia (plus chlorpromazine) and both mammary glands had been prepared for perfusion by separating from the body except for the main artery and vein.

Perfusion experiments. Eight glands were perfused in isolation by the technique of Hardwick & Linzell (1960), as modified by Hardwick, Linzell & Price (1961) and Hardwick, Linzell & Mepharm (1963b). Immediately after removal the glands were washed through with Krebs solution (Hardwick & Linzell, 1960) and, where heparin was not used in the perfusion (Expts. 138 and 139), washing was continued until the venous effluent was nearly clear of blood (about 100 ml. of Krebs solution).

The general conditions of the perfusions are indicated in Table 1. The perfusion medium in Expt. 138 was washed red cells suspended in albumin-Krebs solution (Hardwick & Linzell, 1960), and in Expt. 139 it was red cells suspended in goat serum. In all the other perfusions heparin-treated homologous whole blood was used. Bromolysergic acid diethyl amide (1 mg./l.) was always added to the perfusion medium. All glands were given a substrate solution (glucose, acetate and amino acids) at a rate appropriate to the rate of milk formation. In addition, in Expts. 138, 139 and 140, chyle or washed chylomicra were run into the perfusion medium continuously for 2.5-11 hr. at a rate calculated to provide the gland with enough fat to form about half the milk fat that it had been forming on the goat before the experiment. In Expts. 138 and 139 the chyle was radio-

Table 1. *Summary of perfusion experiments*

Experimental details are given in the text.

Treatment of goat before removal of glands	Expt. no.							
	138		139		140		143*	144*
Spinal anaesthesia	+		+		+		+	-
^3H -labelled chyle injected	-		-		-		+	+
Shot	-		-		-		+	+
Gland	Left Right		Left Right		Left Right		Right	Left
Heparin in perfusion medium	-	+	-	+	+	+	+	+
^3H -labelled chyle infused	+	-	+	+	+	-	-	-
Duration of infusion (hr.)	2.5	.	6.5	7.5	11	.	.	.

* One gland used for analysis.

† Chyle not radioactive.

† Frozen stored ^3H -labelled chyle used.

§ Washed chylomicra used.

active ($2.3\text{--}10.5\mu\text{C}/100\text{ ml.}$; $1.04\text{--}11.5\mu\text{C/g.}$ of fat). In Expts. 143 and 144, ^3H -labelled chyle was injected into the goats before death, but not infused during perfusion.

Analytical methods. These were as described by Hardwick & Linzell (1960) but with the following additions: total esterified fatty acids by the method of Stern & Shapiro (1953); free fatty acids by the method of Dole (1956) and separation of free fatty acids from triglycerides by the method of Borgström (1952); lipoprotein lipase activities by measuring the release of fatty acids from chyle when incubated with plasma as described by Korn (1959).

After being dried at 95° fat was extracted from tissues and milk in a Soxhlet apparatus with light petroleum (b.p. $40\text{--}60^\circ$)-ether (1:1, v/v). Plasma and chyle were extracted with chloroform-methanol by Folch, Lees & Sloane-Stanley's (1957) method. The radioactivity was estimated in a liquid-scintillation counter (Tricarb, Packard). Radioactivity is expressed as counts/min. or disintegrations/min. in experiments where, for other purposes, ^{14}C -labelled substances were also present and the radioactivity due to ^3H had to be calculated. Extracted lipids were dissolved in toluene containing the phosphors 2,5-diphenyloxazole (4 g./l.) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.01 g./l.). The counting of milk and chyle fat presented no difficulties but considerable interference was encountered with tissue fats. The addition of known amounts of radioactivity as an internal standard ($[^3\text{H}]$ -toluene or $[^3\text{H}]$ -stearic acid) to assess the degree of 'quenching' was only successful with extracts from small amounts of tissue (2 g. or less), and then was in reasonable agreement with the radioactivity as assessed by counting different extracts equivalent to various amounts of tissue and extrapolating the counts/g. to zero amounts of tissue.

Fig. 1. Disappearance of radioactivity from the circulation after the injection of ^3H -labelled chylomicra into four conscious lactating goats. With Jill (a) and Bessie (b) and in Expt. 144 (c), fresh chyle was injected; in Expt. 143 (d), washed chylomicra prepared from frozen chyle were used. Quantities of fat injected (mg./kg.) were: Jill, 17; Bessie, 17; Expt. 144, 19; Expt. 143, 34.5.

RESULTS

Physiological reactions to chylomicra. Neither chyle nor washed chylomicra caused any abnormal reactions when given to isolated perfused mammary glands. Small quantities ($0.1\text{--}1.0\text{ ml.}$) were injected intra-arterially and large quantities (10 ml./hr. for $2\frac{1}{2}\text{--}11\text{ hr.}$) were infused into the blood reservoir without any significant alteration in blood flow. Likewise in conscious goats the rapid injection of $35\text{--}60\text{ ml.}$ of chyle containing $1\text{--}2\text{ g.}$ of fat had no noticeable effect. The animals seemed unaware of the injections (made under local anaesthesia) and commenced eating or ruminating when the first few blood samples had been taken. There were no 'acute symptoms' like those recorded by Glascock (1958) in response to emulsified fat in the cow, and the half-life of the radioactivity in the blood was $7.5\text{--}11.5\text{ min.}$ (Fig. 1).

Incorporation of chylomicron lipid into milk fat. This was demonstrated in both the isolated perfused gland and in the conscious goat in experiments with ^3H -labelled chyle that contained over 90% of the radioactivity in the triglyceride fraction. After a single injection of chyle intravenously into two conscious lactating goats (Jill and Bessie) radioactivity appeared in milk fat within 1 hr. and reached peaks at 4 hr. (Fig. 2) and 7 hr. respectively. Within 22 hr. 67% (Jill, Fig. 2) and 38%

Fig. 2. Transfer of ^3H from chyle to milk fat in conscious goat (Jill). The udder volume was 2560 ml. , and the milk yield 123 ml./hr. (equals 100% on graph). ^3H -labelled chyle (50 ml.) containing 2% of fat (1.6×10^4 disintegrations/min./mg. of fat) was injected at 1 hr. The horizontal broken lines for milk composition represent mean ($\pm$ S.D.) concentrations in milk (3 days' combined samples) for Saanen goats in the same herd.

(Bessie) of the injected dose had been recovered in the milk fat.

Chyle labelled with [^3H]stearate was infused in three perfusion experiments; in the first (Expt. 138), which was a trial experiment to determine the feasibility of perfusing without heparin, embolism occurred and the perfusion was not successful. Chyle was infused for 2½ hr. only; nevertheless 7% of the injected dose of radioactivity appeared in the milk fat and the radioactivity/mg. of fat was still rising at the end of the experiment. The two other perfusion experiments with labelled chyle (Expt. 139, left and right glands) were satisfactory. The perfusion medium was washed red cells suspended in serum. The right gland perfused with heparin had 79 ml. of chyle infused during 7.5 hr. and the left gland without heparin had the same quantity of washed chylomicra from the same sample of chyle infused during 6.5 hr. In these experiments also, radioactivity was transferred to milk fat. However, the left gland perfused without heparin incorporated only 0.2% of the injected dose whereas the right gland perfused with heparin-treated blood transferred 28% to milk fat; this difference was partly due to the fact that the left (non-heparin-treated) gland became oedematous and the perfusion was stopped at 12 hr., whereas the right gland secreted milk for 22 hr. Nevertheless, during 12 hr. the right gland secreted 6% of the infused radioactive dose into the milk fat, and the maximum radioactivity/mg. of milk fat was 20-fold higher for the gland receiving heparin (Fig. 3). It is not possible from our results to estimate the amount of milk fat derived from the injected chyle from the ratios of the specific radioactivities of precursor and product, because the fatty acid composition of milk is different from that of blood, and we did not measure the specific radioactivities of stearic acid, but that of total fatty acids in blood and milk.

Heparin is known to release a lipoprotein lipase ('clearing factor') *in vivo* so that it was important to assess its effect in the perfused gland also. This was done by measuring the lipase activity of the plasma and the extent to which the infused chylomicra were hydrolysed. In all experiments the lipase activity of the perfusate increased after circulation through the glands whether heparin was used or not, but this increase was greater when heparin was used. The concentration of free fatty acids in the perfusion medium was raised while chyle or chylomicra were being infused, but the radioactivity of the free fatty acids/ml. of plasma was always much less than that of triglycerides.

In Expts. 138 and 140 chyle was given to one gland but not to the other gland of the same animal perfused at the same time in a separate apparatus. In one case the gland receiving chyle secreted more

fat than the control but the concentration in the milk was lower; in the other, less fat was secreted but the concentration was higher than in the milk secreted by the control. Therefore, though it is clear that chyle is a suitable form of fat emulsion to use in perfusion experiments, since the lipid is incorporated into milk fat, the present experiments do not clearly indicate that triglycerides are an essential component of the substrate mixture for milk secretion under our conditions.

Distribution of injected chylomicron fat. The large percentage of radioactivity that appeared in the milk as a single peak after a single intravenous injection of labelled chylomicra in the conscious animal (Fig. 2) suggested a substantial direct uptake of chylomicra by the mammary gland. The rate of disappearance from the blood (Fig. 1) indicated that this uptake occurred during the first hour or so, and that the delay of some hours between the disappearance from the blood and the appearance in the milk probably represented the period of time necessary for synthesis and secretion of milk fat within the mammary tissue itself (Hardwick, Linzell & Mepharn, 1963*a*). However, the possibility that appreciable amounts of radioactive fatty acid were made available to the mammary gland in a different form as a result of

Fig. 3. Perfusion of right mammary gland, weighing 255 g. and yielding 10.8 ml. of milk/hr. in the 7 days before the experiment (equals 100% on graph), with red blood cells in serum plus heparin (10 i.u./ml.). A substrate mixture of glucose, acetate and amino acids was infused continuously after the second hour, and 79 ml. of goat chyle (2.5×10^4 disintegrations/min./mg. of fat) from 4 to 11.5 hr. The mean blood flow during the perfusion was 54 ml./100 g./min. The horizontal broken lines for milk composition represent the average concentrations for 3 days before the experiment.

Table 2. *Distribution of radioactivity at death in female goats killed after an intravenous injection of ^3H -labelled chylomicra*

In Expts. 144 and G. 184 fresh whole chyle was injected, and in Expt. 143 washed chylomicra prepared from chyle kept frozen for $3\frac{1}{2}$ months, and suspended in the original volume of 0.9% NaCl. The goats in Expts. 143 and 144 were lactating.

	Expt. no.					
	143		144		G. 184	
Weight of goat (kg.)	53		51		50	
Time killed (min.)	21		16		21	
Weight of liver (g.)	1140		1000		850	
Weight of udder (g.)	1180		1500		340	
Weight of omentum (g.)	.		2500		3400	
Weight of fat injected (g.)	1.83		0.98		1.13	
	$10^{-3} \times$	Percentage	$10^{-3} \times$	Percentage	$10^{-3} \times$	Percentage
	counts/min.	of injected	counts/min.	of injected	counts/min.	of injected
Radioactivity injected	2230	100	3500	100	23000	100
Radioactivity in plasma	300	13.5	700	20	2500	11
Radioactivity in liver	700	31	400	11.5	1700-3100	7-13.5
Radioactivity in udder	431	19	1750	50	236-311	1-1.3
Radioactivity in omentum	.	.	300	9	3870-7960	17-34.5
Radioactivity in milk secreted by perfused half udder	56.4	2.5	144.3	4.1	.	.
Length of perfusion (hr.)	21	.	12	.	.	.

uptake and conversion of the chylomicra into other lipid complexes by organs such as the liver cannot be overlooked. We therefore examined in Expts. 144, 143 and G. 184 the distribution of activity between blood, liver and udder of three goats killed 16-21 min. after the intravenous injection of ^3H -labelled chylomicra.

The summary of the results given in Table 2 shows that, as expected from the half-life determinations in the plasma, at 16 and 21 min. after injection only 11-20% of the injected radioactivity was in the plasma. With the injection of fresh chyle into a lactating goat (Expt. 144) half of the injected radioactivity was in the mammary tissue 16 min. later and only 11% in the liver. In Expt. 143 the chyle had been kept frozen for $3\frac{1}{2}$ months and the chylomicra were injected after being washed and resuspended in 0.9% sodium chloride. Resuspension was not fully successful and disappearance from the plasma followed a complex course (first half-life about 2 min.), suggesting that some of the material was unphysiological (Fig. 1). This may account for the finding that in this experiment 31% of the injected radioactivity was recovered in the liver and only 19% in the udder. In both experiments one half of the udder was subsequently perfused in isolation with homologous blood and unlabelled substrates, and 2.5% (Expt. 143) and 4% (Expt. 144) of the total radioactivity injected into the animal was recovered in the milk secreted *in vitro*. The perfusion was technically unsatisfactory in Expt. 144, but in Expt. 143 29% of the radioactivity calculated to be in the tissue at the start of perfusion was recovered in the milk in

Fig. 4. Perfusion of one mammary gland in Expt. 143, where the goat was given ^3H -labelled chylomicra 21 min. before death, and only unlabelled substrates during the perfusion. The gland weighed 540 g. and was yielding 30.4 ml. of milk/hr. before perfusion. The symbols are as in Fig. 3.

22 hr. As in the conscious animal the peak specific radioactivity was reached at about 4 hr. (Fig. 4). In the non-lactating goat (Expt. G. 184) the radioactivity recovered at death was less uniformly distributed within the organs than in the lactating animals. However, 7-13.5% of the radioactivity injected was calculated to be in the liver and only 1-1.3% in the udder. This non-lactating gland contained about 80% of adipose tissue, and 58-

76 % of the total radioactivity in the organ was present in this fat; there was very little adipose tissue in the lactating mammary glands.

Contrary to our previous findings (Hardwick & Linzell, 1960), glands removed from shot animals secreted as much milk during perfusion as glands taken from anaesthetized animals. This may be due to a general improvement in technique, or the more rapid and thorough cooling of the glands after removal from the shot goats in the present experiments, or both.

DISCUSSION

Our results show that chyle may be given in quantity to the isolated perfused gland without blocking the vessels or causing haemolysis. In the conscious lactating animal it was also well tolerated and the half-life of radioactivity in the circulation was of the same order as in other animals. In both the rat and the human large quantities of injected fat are removed from the circulation more slowly than smaller quantities (French & Morris, 1957; Bierman & Hamlin, 1962). Owing to the low specific radioactivity of our chyle, we had to inject 20–40 mg. of fat/kg. body wt. However, Bierman & Hamlin (1962) observed a half-life of 8.7 ± 0.2 min. when a similar amount of chylomicron fat was injected intravenously in man, an animal of similar size to the goat. Their value was 4.3 ± 0.7 min. when only a few milligrams of fat were injected, which may be compared with the half-life of 3 min. calculated by Glascock *et al.* (1957) for 300 mg. of the artificial emulsion in a 460 kg. cow.

The present results also show that the fatty acids of the chylomicra are most probably being continually incorporated directly into milk fat by the udder. This is in agreement with Glascock *et al.* (1957) and Glascock (1958), who found that 20 % of the injected fat appeared in the milk within 90 hr., in spite of the unphysiological nature of the material; a parallel experiment by these authors giving labelled fat by mouth gave similar but less conclusive results, because the peak of radioactivity of blood fat was later than that of the milk fat. In our experiments the peak of radioactivity in milk fat in two experiments occurred at 4 and 7 hr. but did not intersect the descending curve of plasma specific radioactivity. These specific radioactivities are not, of course, true specific radioactivities because they are not measured in terms of stearic acid concentrations in various compounds. Further, it is not possible to apply the argument of Zilversmit *et al.* (1943) because of the existence of large pools of triglyceride intermediates in the udder tissue (Hardwick *et al.* 1963*a*) which delay transport and secretion, so that milk appears at the teat a considerable time after formation of the fat.

Work in the whole animal is not conclusive,

because possible transformations in other organs are not excluded, but this difficulty is overcome in perfusion experiments. In such experiments 28 % of the [^3H]stearate offered can appear in the milk within 22 hr., and in two conscious animals, within the same period, 38–67 % of the injected radioactivity was transferred to the milk fat. The higher degree of incorporation of labelled fatty acids into the milk fat of the conscious goats may be due to the lower efficiency of the perfused gland, or to the transformations of the chylomicron fat in other organs such as the liver and retransport of the labelled fatty acid in a form that is more readily taken up by the mammary gland.

That lactating mammary tissue normally rapidly takes up considerable quantities of chylomicron lipid was clearly shown in the two experiments where lactating goats were killed shortly after an intravenous injection of ^3H -labelled chylomicra, when the blood concentration was expected to be 20–25 % of the initial value. At this time the udder contained up to 50 % of the injected radioactivity and, with fresh chyle, this was considerably more than the liver, the organ usually considered to be the main one for removing chylomicra from the blood in non-lactating animals. This should not be surprising because the udder of the goat is as large as or larger than the liver, and these two animals were forming 69 and 78 mg. of milk fat/min. Barry, Bartley, Linzell & Robinson (1963) reported an arteriovenous difference of 9–21 mg./100 ml. for chylomicron and low-density lipoprotein fat in lactating goats; this is over 50 % of the arterial concentration (76 % for the goat that transferred 67 % of the radioactivity to milk fat in 22 hr. in the present experiments).

The method of uptake of fatty acid from blood triglycerides, however, is still unknown. Lipoprotein lipase is known to be liberated by various tissues after the administration of heparin. There is a marked increase in lipase activity of mammary tissue in the lactating state as compared with the non-lactating state (Robinson, 1963), and both the normal goat udder (Barry *et al.* 1963) and the perfused gland liberate this enzyme even without heparin. It seems probable, therefore, that the chylomicra are lipolysed either within the circulation or just outside it and the released fatty acids transferred to the mammary epithelium. The experiments of Laurysens, Verbeke & Peeters (1961) showed that the perfused cow udder can readily take up labelled stearate from the perfusion medium and incorporate it into udder tissue triglyceride. However, since their experiments lasted only 2 hr. and only 0.03 % of their label appeared in the milk fat, it was difficult to assess the quantitative importance of free fatty acids for the formation of milk fat from this experiment.

SUMMARY

1. ^3H -labelled chylomicra were obtained by feeding ^3H -stearic acid to young goats with cannulated intestinal lymphatic ducts. Most of the radioactivity was in the triglyceride fraction.

2. After the intravenous injection of ^3H -labelled chyle into lactating goats the half-life of radioactivity in the blood was 7.5–11.5 min. The milk fat was maximally labelled at 4 and 7 hr., and 67 and 38 % of the total activity was recovered in the milk fat in 22 hr.

3. In isolated perfused goat mammary glands, ^3H -labelled chyle or washed chylomicra were infused in quantity and 28 % of the radioactivity infused appeared in milk fat in 22 hr. in the presence of heparin.

4. In lactating goats killed 16 and 21 min. after an injection of ^3H -labelled chylomicra, 19 and 50 % of the radioactivity was found in the udder; in one gland perfused *in vitro* 29 % of the radioactivity present was transferred to the milk in 21 hr.

5. It is concluded that the udder of the lactating goat rapidly removes a large proportion of chylomicra entering the circulation.

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Variations in the Concentration of Phytic Acid in the Blood of the Domestic Fowl

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The presence of phytic acid (*myo*-inositol hexaphosphate) in the erythrocytes of chicken blood was first reported by Rapoport (1940), and later Rapoport & Guest (1941) showed that, in the 12 species of birds studied, the concentration of phytate phosphorus varied from 49 to 87 mg./100 ml. of erythrocytes. Phytic acid is not present in the blood of fish, amphibia or mammals,

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and among reptiles Rapoport & Guest (1941) found it only in the erythrocytes of turtles. Nothing definite is known about the function of phytic acid in avian blood cells, and the object of the present study was to investigate the effects of age, sex and reproductive activity on the concentration of phytic acid in the blood of chickens in the hope that the results would indicate the lines along which metabolic studies might most profitably proceed.