Robert and Samuel(7) found the alpha lipoprotein fraction of serum to possess strong inhibitory activity. Tolnay and Bagdy(8), working with human, bovine, rabbit, and guinea pig serum showed the presence of macromolecular inhibitors of elastase which were not dialyzable after 72 hours of dialysis against saline and which were not de-activated by heating to 56°C for 30 minutes. In bovine serum the strongest inhibitors were associated with pseudoglobulin and albumin-type proteins. The work of Walford and Schneider(3), utilizing starch-block electrophoresis of human serum, has indicated the presence of an elastase inhibitor at the junction of the alpha-1 globulin and albumin. Others(8,9) have shown that NaCl, KCl, $(NH_4)_2SO_4$, and Cu^{++} ions all possess inhibitory activity for elastase. The method we have described may be applied to measurement of any elastase inhibitor whether it be serum or any fraction isolated from serum. The stability of the orcein dye in n-butyl alcohol permits storage of samples without fear of change in optical density.

From the points shown in Fig. 3, and the mean values shown for each concentration of serum, it is clear that there is sudden increase in percentage inhibition, as the concentration of serum present is increased from 0.05 to 0.1 ml per 5 ml of reaction mixture. Robert and Samuel(7) also, using a spectrophotometric technic, but with a different substrate and different enzyme preparation, have made a similar observation on pooled human serum. We have found that for any given sample of enzyme and substrate, the results obtained with this technic from repeated assays of a single serum sample are very consistent.

Summary. A spectrophotometric method for determination of elastase and elastase-inhibitory activity has been presented, incorporating Sachar's method of enzymatic digestion of an elastin-orcein substrate with subsequent extraction of the orcein dye from solution with n-butyl alcohol. The inhibitory activity of 15 samples of normal female serum and 2 samples of serum obtained from patients with metastatic pelvic carcinoma was assayed.

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Lipid Content of Chick Erythrocytes and Plasma. (27197)

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The present concern with regard to lipid metabolism and its relationship to health has resulted in a growing interest in the lipids of red blood cells. Mancini and Keys(1) have reported values for cholesterol content of human erythrocytes; Vacca *et al.*(2) have presented glyceride values for human and canine red cells and James *et al.*(3) have demonstrated a high rate of lipid synthesis in human erythrocytes. Since the majority of the studies reported on erythrocyte lipids have been conducted with mammals, comparison of values obtained for the lipid content of avian red cells is of interest since the chicken, in contrast to most mammals, has nucleated erythrocytes.

TABLE I. The Lipid Content of Chick Plasma and Red Blood Cells.

	Plasma	RBC
Lipid, mg/100 ml:		
Ester cholesterol	102 ±12*	5.4 ± 4.2*
Free "	63 ±11	142 ±14
Total "	165 ±16	147 ±15
Lipid phosphorus	8.92 ± 1.39	21.77 ± 2.49
Glycerides (mM/l)	.91 ± .19	.33 ± .05
Total lipids†	539 ±36	725 ±68

* Mean of 10 determinations ± stand. dev.
† Sum of free cholesterol, ester cholesterol × 1.68, lipid phosphorus × 25 and glycerides × 88.5.

Methods. New Hampshire male chicks, fed a stock diet for 5 weeks, were bled by cardiac puncture. Heparin was used as an anticoagulant. An aliquot of blood was used for microhematocrit determination. The plasma was separated from the cells by centrifugation; the cells were washed with normal saline and finally hemolyzed with distilled water. The lipids in plasma and cell hemolysates were extracted with chloroform: methanol (2:1). The lipid extracts were fractionated by silicic acid chromatography(4) following which the appropriate eluted fractions were analyzed for cholesterol by the method of Searcy and Bergquist(5), lipid phosphorus(6), and glycerides by a modification of the method of Van Handel and Zilversmit(7) as previously described(8).

Total lipids were calculated as the sum of free cholesterol, ester cholesterol × 1.68, lipid phosphorus × 25, and glycerides (in mM/l) × 88.5. The factors of 1.68 and 88.5 used to convert ester cholesterol to cholesterol esters and glycerides (in mM/l) to mg/100 ml, respectively, are based on the molecular weight of cholesteryl oleate and trioleate. The factor of 25 employed in conversion of lipid phosphorus to phospholipid assumes an average phosphorus content of 4%.

Results and discussion. The results obtained for the lipid composition of chick plasma and red cells are presented in Table I. The almost complete absence of ester cholesterol in the red blood cell lipids is readily evident, 96.3% of total cholesterol being present as free cholesterol.

This observation is in accord with previous reports for both the human and chicken(9, 10). The total cholesterol for cell lipids listed in Table I is considerably lower than that reported for the chicken by Erickson *et al.*(10), but is similar to the level reported by Mancini and Keys(1) for human cells.

The lipid phosphorus content of cell lipids was more than twice that of plasma, and when converted to phospholipid (× 25) accounted for 75% of the total lipids (Table I). Although the absolute amount found is greater than that previously reported for the chicken(10), the value expressed as a percentage of total lipid is identical. This value noted for phospholipid, 75% of total lipid, is higher than that previously reported for human cell lipids(10).

The values presented here for cell glycerides are lower than those reported by Vacca *et al.*(2) for the dog and man. However, such deviations can undoubtedly be attributed to species variations. The glyceride level is also lower than values for cell neutral fat previously reported for the chicken (10); however, the latter values were determined by an indirect method as compared to the direct method employed in the present study.

An exchange of free cholesterol between cells and plasma has been demonstrated(11, 12); however, only a limited amount of free cholesterol appears capable of entering the cell. This concept is in accord with the observations of Mancini and Keys(1) who have shown that in human subjects dietary variations resulting in plasma cholesterol changes do not alter the cholesterol content of erythrocytes. The results of the present study are also in agreement with the above mentioned observations since no significant correlation ($r = -0.11$) was found between plasma and erythrocyte cholesterol levels. These data indicate that cholesterol metabolism in the nucleated avian erythrocyte is similar to that in the mammalian cell. The lack of correlation between red cell and plasma glyceride levels ($r = 0.40$), noted in this study, supports the observations of Vacca *et al.*(2) that the level of erythrocyte glyceride remains relatively constant in dogs and humans fol-

lowing fatty meals or administration of intravenous fat emulsions. This observation demonstrates a further similarity in lipid metabolism between avian and mammalian erythrocytes.

The data presented indicate that the lipid content of the avian erythrocyte, like the mammalian cell, remains relatively constant and unaffected by plasma lipid levels.

Summary. Data are presented on the composition of chick plasma and erythrocyte lipids. The erythrocyte lipids differ from plasma lipids in that 96% of the cholesterol is present as the free form, lipid phosphorus is over twice as high as the plasma level, and the glyceride level is approximately one-third that of plasma. The total lipid level of red cells (mg/100 ml) was also found to be higher than that of plasma. A lack of correlation between erythrocyte and plasma levels of cholesterol and glycerides was noted, indicating that erythrocyte lipid levels are independent of plasma concentration. The similarities between avian and mammalian erythrocyte lipids are discussed.

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Effect of Repeated Oral Administration of Monoamine Oxidase Inhibitors On Rat Brain Amines. (27198)

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Green and Erickson(1) recently reported that changes in rat brain serotonin (5 HT) concentration produced by a single oral administration of either trans-2-phenylcyclopropylamine hydrochloride (tranylcypromine) or 1-isonicotinyl-2-isopropyl hydrazine phosphate (iproniazid) closely followed the effect of the drug upon brain monoamine oxidase (MAO) activity; *i.e.,* 5HT concentration continued to rise during the time that MAO activity decreased and was completely inhibited, and then returned to normal at about the same rate that the normal enzyme activity was restored.

The time course of changes in brain norepinephrine (NE) concentration induced by iproniazid also reflected the effect of the drug upon MAO activity. Following oral administration of tranylcypromine, however, brain NE concentration reached a peak in about 5 hours then slowly fell during the next 11 hours, while MAO activity remained completely inhibited. This disparity between inhibition of MAO and decrease in elevated NE concentration suggested that an alternate metabolic pathway, in addition to that of MAO, may operate in the rat brain for the degradation of NE, although conclusive evidence in support of this is lacking(2).

The purpose of the present study was to