Table 1. Rf values of 3-hydroxykynurenine obtained with various solvent systems as developer.

Solvent systems, samples	Butanol, acetic acid, water (4: 1: 5)	Butanol saturated with 1-percent NH_4OH	70-percent isopropanol	Methanol, butanol, benzene, water (4: 2: 2: 2)
3-Hydroxykynurenine fraction obtained from the urine	0.38	0.02	0.30	0.36
Pure 3-hydroxykynurenine	.37	.06	.29	.36

The ultraviolet absorption spectrum of this purified sample was the same as that of pure 3-hydroxykynurenine, showing $\lambda_{max.}$ at 370 mμ at *p*H 7.3, which shifted to 310 mμ at *p*H 1.0. The injection of pyridoxine to the diazo-positive patients did not influence the excretion of 3-hydroxykynurenine in urine (*4*).

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Enzymatic Conversion of δ-Amino Levulinic Acid to Porphobilinogen

Porphobilinogen, a compound isolated from the urine of patients with acute porphyria (*1*) has been shown by Bogorad and Granick (*2*) to be converted to protoporphyrin by colorless extracts of *Chlorella* cells. This experiment signifies that enzymes carrying out the conversion of porphobilinogen to protoporphyrin are water-soluble and, thus, that the individual enzymes ought to be amenable to the customary procedures of isolation. Porphobilinogen can also be converted to protoporphyrin by hemolyzates of chicken and red blood cells, as was shown by Falk, Dresel, and Rimington (*3*), and we have confirmed this finding. On the basis of these experiments, porphobilinogen may be considered to be the natural monopyrrole precursor of protoporphyrin.

The precursor of porphobilinogen itself appears to be δ-amino levulinic acid, a compound shown by Shemin and Russell (*4*) to be converted to protoporphyrin by duck erythrocytes. We have found that δ-amino levulinic acid is converted to porphyrins by extracts of *Chlorella* cells, by extracts of spinach, by a strain of *Tetrahymena geleii,* and by extracts of chicken erythrocytes.

Dresel and Falk (*5*) have recently observed the conversion of δ-amino levulinic acid to porphobilinogen by hemolyzed chicken erythrocytes. Recently we have obtained an extract from chicken erythrocytes that converts δ-amino levulinic acid to prophobilinogen. From washed erythrocytes, which have been hemolyzed with water and the hemoglobin denatured with $CHCl_3$-ethanol, a filtrate has been obtained that contains the active enzyme. This aqueous extract is almost colorless. Furthermore, alkaline extracts of the residue contain enzymes that convert porphobilinogen to porphyrins.

The enzyme in the aqueous extract sediments in the ultracentrifuge somewhat more rapidly than does hemoglobin. It may be separated from the accompanying ferritin by starch electrophoresis and appears to be colorless. No appreciable loss of activity accompanies dialysis, so that no loosely bound coenzyme appears to be involved in its activity. Its maximum activity is at *p*H 6.5. The enzyme preparation is somewhat unstable, half of its activity being lost in a week at icebox temperature. Activity is likewise lost during starch electrophoresis in Veronal buffer. In one preparation, δ-amino levulinic acid was observed to be converted to porphobilinogen to the extent of 90 percent of the theoretical as determined with the Ehrlich reagent. The porphobilinogen thus formed has an R_f value in a butanol-acetic-water mixture identical with crystalline porphobilinogen.

The condensation of two molecules of δ-amino levulinic acid to form porphobilinogen requires that two bonds be formed (Fig. 1), a carbon-carbon bond at *A* and a carbon-nitrogen bond at *B,* with the simultaneous removal of two water molecules. One might therefore consider that two enzymes may be involved in this condensation. This does not appear likely since the activity of the enzyme was found to be directly proportional to its concentration through a tenfold dilution of the enzyme preparation. Starch electrophoresis indicated one peak of activity, likewise suggesting that only one enzyme was involved. Whether one enzyme simultaneously brings about the aldol condensation at *A* and the ketimine condensation at *B* remains to be determined. However, since enzymes in general appear to have only one specific activity, it

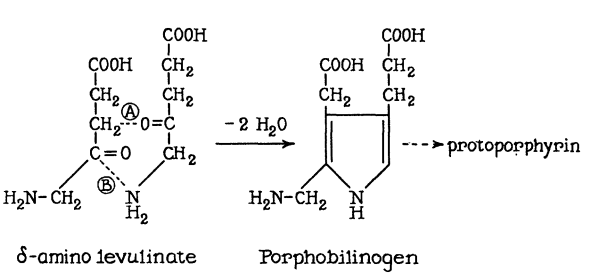

Fig. 1. Structure of porphobilinogen.

would seem more likely that the enzyme catalyzes the aldol condensation at *A*, and that the ketimine condensation then occurs spontaneously at *B*. It may be well to defer naming the enzyme until the specificity of its action has been better defined.

Summary. An enzyme has been found in extracts of chicken erythrocytes which converts δ-amino levulinic acid to the monopyrrole porphobilinogen. Some of its properties have been described (*6*).

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Natural Antioxidants of *Hevea* Latex

Several attempts have been made to identify the constituents of *Hevea* latex responsible for the resistance of raw rubber to oxidation. The most recent of these was that of R. F. A. Altman (*1*) who gives references to earlier work.

No single compound has been clearly shown to be an antioxidant. Altman and others have concluded that protein fragments give some protection against oxidation. Altman, however, found the highest antioxidant activity in an unidentified water-soluble fraction containing no nitrogen.

It is well known that the antioxidant remains in the aqueous phase in ammonia-preserved latex and can be removed by repeated dilution and reconcentration of the latex. The recent observation by M. W. Rider that the antioxidant activity can be removed from latex by a strong base exchange resin (*2*) suggests that the water-soluble fraction obtained by Altman contains an acid (or acids). This suggestion was tested by determining the effect of crude fractions from the serum of a commercial latex on air oxidation, when they were added separately to a sample of the latex that had been treated with strong base exchange resin (*3*).

Latex, stabilized with Antarox D100 (the resin removes soap), was diluted to 20 percent total solids and divided into three portions. One was reserved as a control. The second was passed twice over a column of freshly regenerated strong base resin (Dowex 2), removing serum acids and amino acids. Rubber-free serum was obtained from the third by creaming with 0.15 percent Superloid (ammonium alginate).

Table 1. Viscosities after 17 hr at 110°C.

Rubber from	"Gel" (%)	Intrinsic viscosity
Untreated latex	10	3.21
Dowex treated (A)	4	1.44
A + acid fraction	13	2.75
A + amino-acid fraction	21	2.54
(Unoxidized rubber	7	5.00)

Protein was removed from this serum by adjusting to *p*H 4 with a strong acid resin (Dowex 50) and filtering. Amino acids were collected on a column of Dowex 50, other acids on a weak base resin (Amberlite 1R45).

The 5 percent ammonia eluates from the columns were added to separate portions of the resin-treated latex to give the same concentration originally present. Oxidation resistance of each mixture was determined by spreading 10 ml of the latex in a petri dish, heating in a circulating air oven at 110°C, and measuring intrinsic viscosity of the rubber in chloroform. The results are shown in Table 1.

Strong antioxidant activity is associated with the acid fraction. This has been shown to consist largely of plant acids (*2*). C. E. Rhines (*4*) has presented good evidence that the ease of oxidation of raw crude rubber from which natural antioxidants are removed depends on transition elements, always present in small amounts. Their effect on oxidation is strongly inhibited by the powerful chelating agent, ethylene bis (iminodiacetic) acid. Plant acids apparently have the same function, although they are less efficient. This may explain an earlier observation (*5*) that the rate of oxidation of ammonia-preserved latex decreases with age of the latex. Fresh latex contains little free acid. Hydrolysis of the esters present causes it to appear slowly on storage.

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