each successive addition of carbon monoxide, the percentage conversion of ethylene to ethane was reduced while the carbon monoxide was retained on the surface. The results (Table 4) represent a partial titration of the active surface. It is seen that the catalytic activity for ethylene hydrogenation is proportional to the percentage of surface free from carbon monoxide. Thus there is no evidence of special active sites in the smallest particle (55 Å) examined.

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Thyroid Hormone Control of Erythrocyte 2,3-Diphosphoglyceric Acid Concentrations

Abstract. *A biphasic thyroid hormonal effect has been shown on 2,3-diphosphoglyceric acid synthesis in a crude enzyme, hemoglobin-free preparation from normal human erythrocytes.*

An increase in dissociation of oxygen from hemoglobin occurs on the addition of 2,3-diphosphoglyceric acid (2,3-DPG) to hemoglobin in free solution (*1*). A decrease in the oxygen affinity of hemoglobin in blood of hyperthyroid patients as well as in euthyroid man and rats occurs after treatment with 3,5,3-triiodo-L-thyronine (T3) (*2*). These findings could explain the shift to the right in the oxygen dissociation curve with a subsequent increase in oxygenation of the peripheral tissues. Recently, 2,3-DPG has been shown to be increased in patients with hyperthyroidism (*3*) as well as in normal red cells incubated with T3 (*4*).

To investigate whether the thyroid hormonal effect on intact cells was on the red cell membrane, on a specific enzyme, or on a group of enzymes, a crude enzyme preparation from human erythrocytes was prepared. Seven-day-old whole blood (200 ml), collected in ACD (citric acid, trisodium citrate, and dextrose), was washed three times in ice-cold saline; the packed cells were adjusted to a hematocrit of 35 to 40 percent with a Kreb's-Ringer bicarbonate buffer (*p*H 7.4) containing $10^{-4}M$ mercaptoethanol and $10^{-5}M$ ethylenediaminetetraacetate. These cells were frozen and thawed three times, and the stroma were removed by centrifugation at 30,000*g* for 30 minutes. The stroma-free hemolysate was then mixed with an equal volume of a suspension of 10 percent diethylaminoethyl (DEAE) cellulose in 3 mM potassium phosphate buffer (*p*H 7.26); the mixture was allowed to sit at 4°C for 30 minutes and was then centrifuged. The hemolysate was discarded and the DEAE-cellulose suspension was repeatedly washed with an equal volume 3 mM potassium phosphate buffer (*p*H 7.26) until the supernatant was colorless. The enzymes were eluted from the resin with 0.5M KCl (two 50-ml volumes). The supernatant, free of hemoglobin and rich in enzyme, was then dialyzed overnight against 0.15M KCl (*p*H 7.26) containing $10^{-4}M$ mercaptoethanol. The following day 2.5 mg of bovine serum albumin per milliliter of partially purified enzyme fraction was added, and diphosphoglyceromutase was assayed (*5*). The enzymatic assay for 2,3-DPG was performed by Beutler's modification (*6*) of Krimsky's method (*7*); 1,3-diphosphoglyceric acid and 3-phosphoglyceric acid were assayed by the methods of Negelein (*8*) and were either absent or not detectable. In the crude enzyme preparation, the 2,3-DPG concentrations were 5 to 7 nmole/ml.

The crude enzyme preparation containing diphosphoglyceromutase was incubated in a system which generated 1,3-diphosphoglyceric acid. The system contained (in a total volume of 2.2 ml) glyceraldehyde-3-phosphate (20 μmole), nicotinamide-adenine dinucleotide (10 μmole), 3-phosphoglyceric acid (0.1 μmole), $MnCl_2$ (1 μmole), potassium phosphate (*p*H 7.5) (2 μmole), lactic dehydrogenase [4 international units (I. U.)], potassium pyruvate (40 μmole), glyceraldehyde-3-phosphate dehydrogenase (5 I.U.), triethanolamine-hydrochloride buffer (*p*H 7.8) (100 μmole), and mercaptoethanol ($10^{-4}M$), with a final *p*H of 7.6 to 7.7. Varying concentrations of L-thyroxine (T4) and T3 were added, and the preparation was incubated for 1 hour (Fig. 1). Stock solutions of the thyroid hormones were prepared by diluting T4 or T3 in water distilled twice in glass just before use to a concentration of $3.75 \times 10^{-4}M$. A few drops of 1N NaOH were added, and the solution was heated to 40°C to increase solubility. Subsequent dilutions were made from this stock. The results show a biphasic effect on 2,3-DPG synthesis. The stimulatory effect was first witnessed with T3 at concentrations of $3.75 \times 10^{-14}M$ and was maximum at $3.75 \times 10^{-8}M$; the effect decreased with increasing concentrations of thyroid hormone. The effect of T4 was noted initially at a concentration of $3.75 \times 10^{-12}M$ and peaked at $3.75 \times 10^{-10}M$.

Astrup *et al.* (*9*) recently pointed out that no biochemical explanation has been presented for the shifts in oxygen dissociation curve observed in thyroid disorders by Gahlenbeck and Bartels (*2*).

We have demonstrated a direct effect on the concentration of 2,3-DPG in the red cells by thyroid hormone. In view of previous findings (*1*) in conjunction with those discussed above, a biochem-

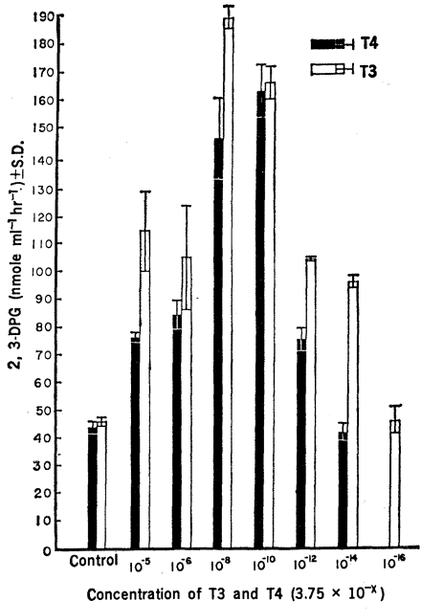

Fig. 1. The effect of thyroid hormone [3,5,3-triiodo-L-thyronine (T3) and L-thyroxine (T4)] concentration of 2,3-diphosphoglyceric acid synthesis in a 1,3-diphosphoglyceric acid generating system and a crude erythrocyte enzyme preparation as compared to control ($N = 4$).

ical explanation for the altered oxyhemoglobin dissociation curve in thyroid disease is now available. Thyroid hormone increases 2,3-DPG concentration with a subsequent shift to the right. The exact molecular mechanisms of the interaction of the thyroid hormone and erythrocyte 2,3-DPG synthesis remain to be determined.

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Desmosterol as the Major Sterol in L-Cell Mouse Fibroblasts Grown in Sterol-Free Culture Medium

Abstract. *The principal sterol synthesized by L-cell mouse fibroblasts is desmosterol. Cholesterol was not detected in these cells when they were grown in a sterol-free culture medium. These findings indicate that, in cells, cholesterol can be replaced by desmosterol. Sterol analyses of six other tissue culture cell lines revealed cholesterol synthesis.*

A number of investigations have shown that many tissue culture lines and strains have the potential to synthesize sterol from precursors, such as glucose, mevalonate, and acetate (*1–5*). It has been assumed that the major sterol synthesized by those cells is cholesterol; however, in only a few of these studies has a detailed identification of the nature of the sterols been attempted. Evidence will be presented that one of the cell lines studied in the present experiment, L-cell mouse fibroblasts, does not synthesize cholesterol, but rather two sterols differing from cholesterol in chromatographic behavior and in other physical characteristics. The major steroidal component isolated from these cells has been identified as desmosterol.

The L cell line used in this study has been maintained in this laboratory for more than 3 years and has been grown in medium containing delipidized calf serum protein for approximately 2 years (*5*). For comparative purposes a second L cell line (L-929) was purchased from Flow Laboratories, Inc., Rockville, Md. Other cell cultures used in this study were: WI-38 human diploid embryonic lung cells and WI-38VA13, a line of WI-38 cells transformed by simian virus 40, obtained from Dr. V. Cristofalo, Wistar Institute; HEp-2 human carcinoma and KB human carcinoma, purchased from Flow Laboratories; the Nil-2 cell line of spontaneously transformed hamster cells, from Dr. L. Diamond, Wistar Institute; and the ST-3 line of murine adenocarcinoma, from Dr. P. Koldovsky, Wistar Institute.

All cells were grown for a minimum of three passages (2 to 3 weeks) in a lipid-free medium before sterol analyses were conducted. The growth medium in all cases was Eagle medium containing double-strength amino acids and vitamins in Earle balanced salt solution, supplemented with 5 to 10 mg/ml delipidized calf serum protein (*5*). Two to four milk dilution bottles with confluent cell monolayers provided sufficient material for the sterol analyses by thin-layer chromatographic (TLC) methods. When larger quantities of sterol were desired, cells were obtained from eight to ten 2-liter Blake bottles. In all analyses the cellular sterol was labeled isotopically by the addition of [2-^{14}C]sodium acetate (24.5 μc/mmole) to the culture medium at a level of 250 μg/ml. The labeled acetate was added at the time of inoculation and was present throughout the cellular growth cycle (5 to 7 days). Cells were treated with trypsin and isolated as previously described (*5*).

Methods for the extraction, saponification, and digitonin precipitation of the sterol used for TLC analysis have been described in a previous paper (*5*). The digitonin-precipitable material was obtained after splitting the digitonides with dimethyl sulfoxide (*6*), and these sterols were resolved by TLC with the use of silver nitrate–silica gel H plates developed with a mixture of $CHCl_3$ and acetone (95 : 5, by volume) (*7*). Radioactive regions were detected by the use of a Packard radiochromatogram scanner 7201. L cells were the only cell line studied that, when grown in the absence of exogenous sterol, contained a significant amount of a sterol with TLC properties differing from those of cholesterol. Since this TLC system is capable of separating a wide variety of commonly occurring sterols (*7*), only the material obtained from L cells was examined in greater detail.

The following methods were used to purify the major sterol synthesized by the L cells. Extraction, column separation of phospholipids from neutral lipids, and saponification of neutral lipids were performed according to the procedures described by Conner *et al.* (*8*); free sterol was acetylated by the method of Johnston *et al.* (*9*); acetylated sterols were purified on silver nitrate–Unisil columns (*10*); and TLC studies of acetylated sterols were carried out on silver nitrate–silica gel H plates developed with a mixture of benzene and hexane (3 : 5, by volume) (*10*).