are required for this purpose because the energy of the monochromatic light emerging must be considerable to illuminate effectively even small plates.

Using a hydrogen lamp, substances which maximally absorption at 207 mμ such as cholesterol and 7α-hydroxycholesterol, can easily be detected. Thus by using a series of interference filters in the range 200–300 mμ with a hydrogen source, this method not only shows the position of a substance on the plate but indicates its ultraviolet-absorbing properties and thus gives information on its possible molecular structure.

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The effect of thyroid hormones on oxygen consumption of isolated horse leucocytes

As part of a continuing study of the effects of thyroid hormones and their attendant molecular alterations at various levels of biological organization, we have studied the oxygen consumption of isolated horse-blood leucocytes. Previous studies have indicated: (1) a correlation of O_2 consumption of human leucocytes with the thyroid status of the donor[1,2] (2) increased leucocyte O_2 consumption after administration *in vivo* of thyroid hormone[3] and (3) response *in vitro* to triiodothyroacetic acid of myeloid leukemic leucocytes[4].

This report deals with a prompt increased O_2 consumption of horse leucocytes by addition *in vitro* of L-triiodothyroacetic acid and a lesser and delayed increase with L-thyroxine and L-triiodothyronine.

Leucocytes were harvested from 4 l of horse blood by the methods of MAUPIN[5], avoiding the addition of any substance other than crystalline heparin, yielding $0.8 \cdot 10^{10}$–$3.2 \cdot 10^{10}$ leucocytes containing less than 1 erythrocyte per 15 leucocytes. The O_2 consumption of $10 \cdot 10^7$–$20 \cdot 10^7$ leucocytes was determined, in triplicate, by standard manometric techniques immediately after preparation and after storage at 0° for 4, 24, and 48 h (expressed as μmoles O_2 consumed per h per 10^{10} leucocytes). Thyroid hormones, solubilized in 2–3 drops of NaOH, were added just before the run or from the side arm after 15-min equilibration.

In Krebs–Ringer phosphate media[6], O_2 consumption ranged from 33.2 to 91.5 μmoles O_2/h/10^{10} leucocytes, averaging 55.0 ± 15.8. Suggestive increases were observed with thyroid hormone addition ($2 \cdot 10^{-6}$–$5 \cdot 10^{-6}$ M) but were small and variable (9 to 22 % with triiodothyroacetic acid, 0–10 % with triiodothyronine or thyroxine).

In subsequent runs using a buffer medium devised by DICKENS AND SALMONY[7] (see Table I) O_2 consumption was strikingly higher (from 98.6–225 μmoles O_2/h/10^{10}

leucocytes in 50 runs, averaging 151.8 ± 35.0). In addition the effects of added thyroid hormones were greater and more consistent with this medium. Triiodothyroacetic acid caused a greater percentage increase than thyroxine or triiodothyronine at each concentration within the effective range. In 31 runs, the addition of triiodothyroacetic acid ($1.2 \cdot 10^{-6}$–$5.6 \cdot 10^{-6}$ M) resulted in increases of 19.0–59.0 %, averaging

TABLE I

EFFECT OF TRIIODOTHYROACETIC ACID ON O_2 CONSUMPTION OF HORSE LEUCOCYTES *in vitro*

Reaction medium: 0.4 ml of 0.22 M phosphate buffer (pH 7.4), 0.3 ml of 0.01 M AMP, 0.1 ml of 0.2 M $MgSO_4$, 0.4 ml of 0.11 M sodium succinate, 0.1 ml of $2 \cdot 10^{-4}$ M cytochrome *c*, 0.3 ml of 0.25 M fructose, 0.3 ml (50 μg) hexokinase, 0.1 ml of 0.39 M NaF, 1 ml leucocytes, 0.3 ml hormone solution, 0.2 ml 10 % KOH in center well.

Triiodothyroacetic acid (M)	*% increase in O_2 consumption*
$5.6 \cdot 10^{-7}$	0
$7.0 \cdot 10^{-7}$	14.4
$1.4 \cdot 10^{-6}$	19.0
$2.8 \cdot 10^{-6}$	43.2
$5.6 \cdot 10^{-6}$	38.0
$5.6 \cdot 10^{-5}$	13.2

34.8 ± 9.2 %. In 2 runs, no increase was found; in 3 runs with control values greater than 200 μmoles/h/10^{10}, increases of 11.4, 12.0 and 17.0 % were noted. Maximal effects were obtained with $2.8 \cdot 10^{-6}$ M triiodothyroacetic acid (Table I). The narrow range of effective concentrations is noteworthy, larger concentrations leading to inhibition below control values.

When triiodothyroacetic acid was added to the medium just before the start of the run, increased consumption was noted at 5–15 min (after 15-min equilibration). When triiodothyroacetic acid was added from the side arm after equilibration, increases were noted in 2 experiments at the 5-min reading, in all others between the 10- and 15-min reading. In most experiments, increases were noted at times when the control rates were sustained at constant levels, indicating an absolute stimulatory effect. In other experiments, this effect could not be clearly separated from that of sustaining the level, as noted by others in other systems[8,9] as control levels per 5 min interval appeared to be declining slightly at the time the triiodothyroacetic acid effect became apparent.

Oxygen consumption was the same at 4 h after leucocytes isolation but decreased progressively at 24 and 48 h, associated with decreased motility on phase microscopy and increasing cellular disruption with liberation of granules. The stimulatory hormone effects similarly decreased progressively (Table II).

In experiments excluding individual components of the DICKENS–SALMONY medium, O_2 consumption was unaffected by omission of AMP, hexokinase, or cytochrome *c*. Omission of Mg^{2+} resulted in a small decrease. The stimulatory effect of triiodothyroacetic acid was unchanged on omission of AMP or cytochrome *c* or by the use of either glutamate or succinate as substrate. When hexokinase was omitted, there was either no change or a slightly decreased effect of triiodothyroacetic acid.

The presence of fluoride (F^-) appeared to exert the major differential effect. Control O_2 consumption, in 10 runs, ranged from 28.0 to 228 % higher in the presence

of F^-, averaging 87.4 %. The stimulatory effects of triiodothyroacetic acid, observed with the entire medium, were either markedly diminished, abolished completely, or reversed in the absence of F^-. The addition of F^- alone to Krebs–Ringer phosphate buffer resulted in clear-cut increases of O_2 consumption at $5 \cdot 10^{-4}$ M, with maximal effects at $5 \cdot 10^{-3}$ M.

TABLE II

EFFECT OF THYROID HORMONES ON O_2 CONSUMPTION OF HORSE LEUCOCYTES *in vitro* WITH INCREASING TIME AFTER ISOLATION

Reaction medium same as for Table I. Each hormone added to final concentration of $5 \cdot 10^{-6}$ M.

Time after isolation (h)	*Control O_2 consumption (μmoles O_2/h/10^{10} leucocytes)*	*% Increase in O_2 consumption*		
		triiodothyroacetic acid	*triiodothyronine*	*thyroxine*
1	150.0	55.7	21.9	13.3
24	124.1	39.6	8.0	8.0
48	79.0	23.6	10.6	13.4

In this system, effects of triiodothyronine and thyroxine were comparable to each other and always less than those of triiodothyroacetic acid (increases of 8–22 % for triiodothyronine, 7–14 % for thyroxine; Table II). The effects also were more delayed, becoming apparent 35–45 min after addition. The effects of triiodothyronine were markedly diminished by substitution of glutamate for succinate. Thus, in an enriched buffer medium containing fluoride, the addition *in vitro* of triiodothyroacetic acid caused a prompt increase in the O_2 consumption of isolated horse leucocytes. Lesser and delayed increases were observed with thyroxine or triiodothyronine.

It may be that this and the other more rapid effects of triiodothyroacetic acid are due to the previously demonstrated more rapid penetration of this derivative into leucocytes, compared with or thyroxine triiodothyronine.

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