parotid fluid. We also wish to thank Miss Suanne Spaeth for technical assistance.

1. Afonsky, D., Saliva and Its Relation to Oral Health, Univ. of Alabama Press, 1961.
2. Landy, M., Weidanz, W. P., Bacterial Endotoxins, M. Landy, W. Brown, ed., Rutgers Univ., 1964, p275.
3. Kraus, F. W., Konno, J., Ann. N. Y. Acad. Sci., 1963, v106, 311.
4. Stoffer, H. R., Kraus, F. W., Holmes, A. C., Proc. Soc. Exp. Biol. and Med., 1962, v111, 467.
5. Tomasi, T. B., Tan, E. M., Solomon, A., Prendergast, R. A., J. Exp. Med., 1965, v121, 101.
6. Brill, N., Bronnestam, R., Acta Odont. Scand., 1960, v18, 95.
7. Mann, W. V., Stoffer, H. R., Periodontics, 1964, v2, 263.
8. Muschel, L. H., Treffers, H. P., J. Immunol., 1956, v76, 1.
9. Weidanz, W. P., Landy, M., Proc. Soc. Exp. Biol. and Med., 1963, v113, 861.
10. Crowle, A. J., Immunodiffusion, Academic Press, New York, 1961.
11. Benacerraf, B. M., Miescher, P., Ann. N. Y. Acad. Sci., 1960, v88, 184.
12. Shilo, M., Brit. J. Exp. Path., 1962, v43, 142.
13. Michael, J. G., Rosen, F. S., J. Exp. Med., 1963, v118, 619.
14. Tung, F. F., Schein, A. H., J. Dent. Res., 1964, v43, 423.
15. Bull. Wld. Hlth. Org., 1964, v30, 447.

Received April 2, 1965. P.S.E.B.M., 1965, v119.

Failure of L-Triiodothyronine to Alter Significantly Glucose Utilization by Human Erythrocytes.§ (30310)

B. CALESNICK (with the technical assistance of M. Richter and G. Weintraub)
Laboratory of Human Pharmacology, Hahnemann Medical College, Philadelphia, Pa.

It was demonstrated that parenteral administration of L-Triiodothyronine (L-T3) initially increased and then decreased utilization of glucose by the RBC of rabbits(1). Because of this fact, we became interested in studying the effects of clinically large doses of this compound on utilization of glucose by the human erythrocytes. Certainly, if a peripheral cellular effect could be demonstrated, then there would be a possibility of establishing a clinically useful tool for reflecting total body metabolism and perhaps evaluating thyroid function. The present investigation was therefore designed to study in humans: 1) normal fluctuations in rate of glucose utilization by RBC, and 2) effect of L-T3 on this rate.

Methods and material. Fasting venous blood was obtained from healthy volunteer men and women to insure satisfactory range in age and sex distribution. These individuals were clinically free of any disease and not taking medication. The laboratory procedure followed has been described(1). Blood samples were collected with siliconized equipment, and heparin was used as the anticoagulant. *In vitro* tests were conducted by centrifuging the samples for 20 minutes at approximately 2700 RPM (1000 *g*'s) in an International Clinical centrifuge. The buffy coats and upper red cell layers were carefully aspirated and discarded and the RBC were then resuspended in the subject's own plasma. The final hematocrit was adjusted to approximately 50%. Preparation of RBC suspensions were conducted at room temperature. White blood counts were done on the original sample of blood and on the final suspension of RBC to estimate efficiency of removal of WBC, because of their relatively high rate of glucose metabolism(2,3,4). Glucose determinations were made in duplicate using the Somogyi-Nelson procedure(5). Within one hour after venipuncture, samples in glass-stoppered centrifuge tubes were incubated at 36-37°C in a water bath and equilibrated in air atmosphere since previous experiments did not reveal a significant difference in glucose metabolism under aerobic and

§ This investigation has been made with the assistance of a grant from the Committee on Research, Council on Drugs, American Medical Assn.

anaerobic conditions. The tubes were shaken gently every 10 minutes to keep the RBC's in suspension, and aliquots for glucose analyses were withdrawn after 10 and 70 minutes. These periods represented the "zero" or control and 1 hour determinations, and the rate of glucose disappearance was assumed to be the RBC utilization rate. The pH of the reconstituted blood was measured with a Corning pH meter model 12, using a combination electrode, at the beginning and end of the incubation period.

Normal fluctuations in rate of glucose utilization by RBC were determined by study of the following groups:

1. A pool of 108 subjects (62 males and 46 females), 22 to 65 years of age, was studied between 1957 and 1964, to establish the mean rate of glucose utilization by the RBC. These subjects consisted mainly of medical students, laboratory technicians, and instructors.

2. A group of these men and women was studied to determine if a correlation exists between blood sugar levels and rate of glucose utilization.

3. Men were studied weekly for approximately 2 months to determine individual variability of the utilization rate.

4. Females were studied to determine any changes in utilization of glucose associated with the normal menstrual cycle.

The definitive study of the effect of L-T3 on the rate of glucose utilization by RBC was performed on a group of 12 men, who were placed on 400 μg per day (100 μg q.i.d.) of L-T3* by mouth as the sole medication in order to product an experimental thyrotoxic state. Medication was discontinued when evidence of marked drug-induced clinical toxicity appeared. The following parameters were monitored periodically during this experimental period: 1) glucose utilization, 2) body weight, 3) blood pressure, 4) pulse rate, and 5) red cell uptake of I^{131} L-T3 using the resin-sponge method.†

Results. The results are expressed as either mg glucose utilized per hour per 100 ml packed red cells or mmols glucose utilized per hour per liter packed red cells.

1. The mean "zero" or control blood sugar for the 108 healthy male and female subjects was 65.4 ± 0.7‡ mg per 100 ml or 3.63 ± 0.04 mmols per liter. The utilization rate was 37.0 ± 1.9 mg glucose per hour per 100 ml packed red cells or 2.05 ± 0.11 mmols glucose per hour per liter packed red cells. The white blood count was reduced from the mean of 8.57 ± 0.21 × 10^3/cmm for the drawn blood to a mean of 1.65 ± 0.11 × 10^3/cmm for the reconstituted blood with a 50% hematocrit. This represented a reduction of approximately 81%. The pH of the reconstituted bloods increased from a mean of 7.57 to 7.64 during the 1-hour incubation.

2. To extend the investigation of possible correlation between blood sugar levels and rate of glucose utilization, 13 randomly selected men and women from the 108 subjects each received 100 g of glucose by mouth. The rate of glucose utilization was determined 2 hours after glucose-loading. In this small group, the mean "zero" or control blood sugars increased from 71.4 ± 0.4 to 101 ± 11 mg per 100 ml and the mean rate of utilization changed from 29.8 ± 0.8 to 29.2 ± 3.8 which was not significant ($P>0.7$).

3. Rates of glucose utilization for a group of 6 men repeated at intervals did not reveal a significant biological rhythmicity (Fig. 1).

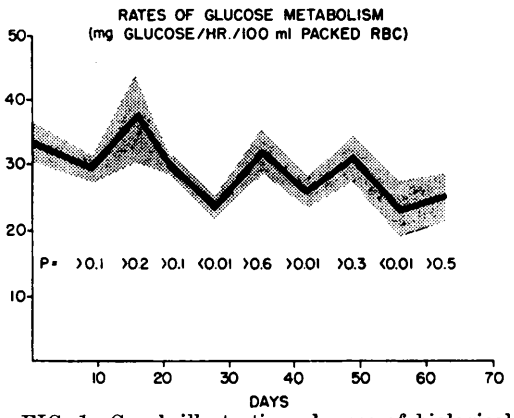

FIG. 1. Graph illustrating absence of biological rhythmicity in glucose utilization by RBC of male subjects. Shaded areas are standard errors.

* Supplied by Smith, Kline & French Laboratories, Philadelphia, Pa., as 100 μg tablets.

† Supplied by Abbott Laboratories, North Chicago, Ill., as Triosorb.

‡ ± S. E.

TABLE I. Summary of Effects Produced by Daily Oral Administration of 400 μg L-Triiodothyronine.

Subj No.	Pulse (rate/min)			Systolic B. P. (mm Hg)			Wt (lb)			Utilization or glycolysis rate (mg glucose per hr per 100 ml packed RBC)			I^{131} L-T3 uptake (% uptake of radioactivity by RBC)			Duration of treatment
	Control	Treated	% change	Control	Treated	% change	Control	Treated	% change	Control	Treated	% change	Control	Treated	% change	Days
1	72	130	80	120	136	13	145	145	0	44	14	−68				21
2	88	124	40	125	148	18	172	162	−6	44	10	−77				27
3	90	126	40	120	134	11	174	164	−6	48	62	29				35
4	84	102	21	110	112	2	160	150	−6	52	66	26	27.2	31.3	15	9
5	72	92	27	108	126	16	194	190	−2	36	48	33	28.8	29.3	2	9
6	116	152	31	110	118	7	167	162	−3	16	12	−25				19
7	92	132	43	118	110	−6	164	160	−2	14	38	171				41
8	96	136	41	105	136	29	220	206	−6	40	78	95				19
9	72	130	80	117	135	15	172	165	−4	22	38	73				21
10	64	100	56	112	116	3	161	153	−5	68	28	−58	37.1	43.7	17	9
11	90	122	35	120	135	12	190	182	−4	26	20	−23				35
12	52	100	92	130	114	−12	149	142	−5	48	66	37	32.2	36.0	11	9
Mean % change			49			9			−4			18			11	

4. In determining rates of glucose metabolism throughout the menstrual cycle, a group of 25 young women of childbearing age, who had no gross irregularities in the rhythmicity of their menstrual cycles, was selected from our pool of 108 subjects. Measurements were made at 5-day intervals for 30 days. No statistically significant changes were observed (Fig. 2). The post-menstrual mean utilization rate was 34.0 ± 1.8 mg glucose per hour per 100 ml packed red cells which was not significantly different from the mean rate of 33.3 ± 2.0 mg glucose per hour per 100 ml packed red cells obtained during menses ($P>0.7$).

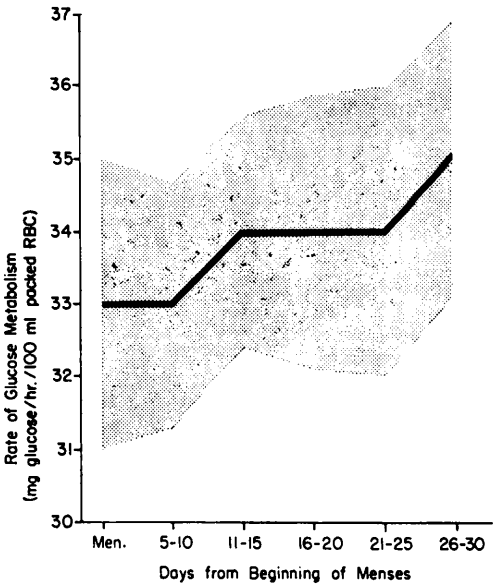

FIG. 2. Non-significant mean changes in rate of glucose metabolism during menstrual cycle. Shaded areas are standard errors.

The changes induced by continuous oral administration of L-T3 are summarized in Table I. Medication was discontinued in each subject when any of the following complaints became intolerable: palpitation, tremors, nervousness, or insomnia. Between 9 and 41 days were required to establish this clinical endpoint for the purpose of this investigation. There were significant increases of mean pulse rate and systolic blood pressure associated with a decrease in mean body weight. The greatest change was reflected in the development of tachycardia. Concomitant findings were an apparent increase in both rate of glucose utilization and uptake of (I^{131}) L-T3 by RBC. However, when these latter mean changes were analyzed statistically, they were minimal and not significant.

Discussion. The rate of glucose utilization was found not to be significantly different between the men and women volunteers, which confirms the findings of other workers(6). Previous investigators have also reported that the level of blood glucose did not affect the rate of glucose utilization and diabetic pa-

tients were reported to have normal rates of glucose utilization(7). On the other hand, ultrafiltrate from patients with uremia were shown to inhibit this rate and this inhibition was demonstrated to be independent of variations in pH or glucose concentrations(8). An increase in blood lactic acid was reported to occur during ovulation(9). However, this might well reflect changes in total body metabolism rather than an RBC metabolism.

The major pathway of glucose metabolism in the RBC is the anaerobic Embden-Meyerhof pathway which results in formation of lactic acid and production of the high energy bonds of ATP. An average of only 11% of the total glucose utilized is metabolized by the aerobic phosphogluconic pathway which results in the formation of CO_2 and the production of potential energy as NADPH(10, 11).

A decrease in blood pH decreases glucose metabolism because of the depletion of 2,3 diphosphoglycerate (2,3-PGA) which is also present in RBC(12).

However, when the pH of blood was increased to approximately 7.8, it has been reported that total glucose utilization was increased by 43% and that this increase was localized in the Embden-Meyerhof pathway (10). We found that when the reconstituted blood samples were equilibrated in a closed system of air-atmosphere, the pH increased by a mean of only 0.07 during the one-hour incubation period. The mean rate of glucose utilization obtained during these experiments was 2.05 $\pm$ 0.11 mmols per hour per liter packed RBC which agrees with the limits of 0.9 to 3.5 mmols glucose per hour per liter established by other investigators(3,11,12).

Under the conditions of this study, as manifested by the individual variabilities, there was no consistently significant change in this mean utilization rate during the administration of L-T3. However, these individuals exhibited clinical evidence of increased general metabolism as shown by tachycardia, increased systolic blood pressure, and loss of body weight. The average daily dose of L-T3 that was administered to these individuals was limited because of toxicity to approximately 5.5 μg per kg. On the other hand, in our animal studies(1), 125 μg per kg per day was the dose used to produce initially approximately a 50% increase in the glucose utilization of the RBC. Since *in vitro* studies have shown that thyroid hormone enhances aerobic metabolism of RBC(13) or can decrease glucose metabolism by depressing the triose phosphate dehydrogenase system(14), perhaps larger doses of this drug might have produced a more significant metabolic change in the RBC of our subjects. It might be assumed that L-T3, in the dose used, did not affect the carrier transport system(15) or the various enzyme systems that control the rate of glucose metabolism in human RBC, and that the increase in total body metabolism is not significantly reflected by the rate of glucose utilization of RBC.

1. Calesnick, B., Altarelli, V. R., Spirtes, M. A., Endocrinology, 1960, v66, 517.

2. Bartlett, G. R., Marlow, A. A., J. Lab. Clin. Med., 1953, v42, 178.

3. Bernstein, R. F., Nature, 1953, v172, 911.

4. Bird, R. M., J. Biol. Chem., 1947, v169, 493.

5. Nelson, N., ibid., 1944, v153, 375.

6. Sartori, S., Valaqussa, L., Palamara, F., Petronio, L., Haematologica, 1961, v46, 581.

7. Haller, H., Strauzenberg, S. E., Coetze, H., in Diabetes; Abstracts of papers presented at Fifth Congress of International Diabetes Federation, Toronto, 1964. Excerpta Med. Int. Congress Series No. 74, 1964, p82.

8. Morgan, J. M., Morgan, R. E., Metabolism, 1964, v13, 629.

9. Duboff, G. S., Behrman, S. J., Saraiya, H., Frohman, C. E., Latham, L. K., Czajkowski, N. P., Obstet. Gynec., 1961, v18, 564.

10. Murphy, J. R., J. Lab. Clin. Med., 1960, v55, 286.

11. Chapman, R. G., J. Clin. Invest., 1962, v41, 1249.

12. Prankerd, T. A. J., The Red Cell, Charles C Thomas, Springfield, Ill., 1961.

13. Necheles, T., Beutler, E., J. Clin. Invest., 1959, v38, 788.

14. Wolff, J., Wolff, E. C., Biochem. et Biophys. Acta, 1957, v26, 387.

15. Lefevre, P. G., Am. J. Physiol., 1962, v203, 286.

Received April 8, 1965. P.S.E.B.M., 1965, v119.