lecithin could be transported to a site of action for release by liberation of the aldehyde. Whether such a site of action would be the heart or some other organ or organs cannot yet be decided. These questions are being investigated further.

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27 September 1956

Evidence for Nonexistence of D-Aspartic Acid in Casein

The values reported previously for L-aspartic acid and total (L + D) aspartic acid in acid hydrolyzed casein (*1*) imply that the aspartic acid in this material was approximately 7 percent racemic (*2*). The question of whether racemization to this extent is a consequence of the hydrolysis procedure or whether D-aspartic acid residues are initially present in intact casein was left unsettled but appears now to be resolved by the results of the present experiments (*3*).

Samples (3 g each) of casein (*4*) were heated with 30-ml portions of 6*N* hydrochloric acid under reflux for varying periods of time, and each hydrolyzate was assayed for L-aspartic acid and for total aspartic acid by the previously described methods (*1*). The L-aspartic acid values (*5*) found after heating for 29, 32, 340, 550, and 720 hours, respectively, were 6.65, 6.92, 4.52, 4.00, and 3.77 percent, whereas the corresponding total aspartic acid values (*5*) averaged 7.21 (7.20, 7.31, 7.15, 7.20, 7.17) percent. Corresponding values for nonracemic aspartic acid (*6*) in each hydrolyzate were calculated from these data, and the logarithms of the resulting values were plotted against time (Fig. 1), revealing the linear relationship that would be expected for racemization at a constant rate. The straight line shown in Fig. 1 is that fitting the data most closely according to the theory of least squares. It extrapolates at zero time to a value corresponding to 7.37 percent nonracemic aspartic acid, a value that agrees within experimental error with the mean value for total aspartic acid

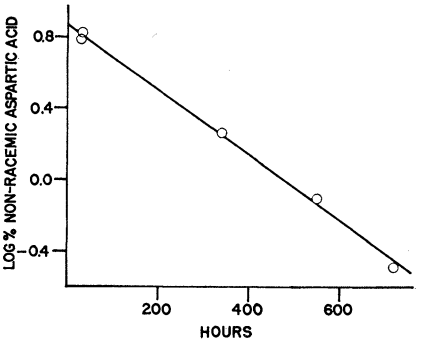

Fig. 1. Nonracemic aspartic acid in casein hydrolyzate as a function of refluxing time.

(7.21 percent). It appears, therefore, that none of the aspartic acid in casein is initially racemic and that racemization of the aspartic acid in casein proceeds essentially at the same rate before and after liberation by acid hydrolysis.

Interpolation on the curve shown in Fig. 1 indicates that the aspartic acid in mixtures of casein and 6*N* hydrochloric acid is 4.1, 8.0, and 11.8 percent racemic, respectively, after 10, 20, and 30 hours of heating the mixtures under reflux.

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2. Mixtures of L- and D-aspartic acids are considered here (for convenience of mathematical treatment) to be mixtures of racemic (the fraction containing equal amounts of L- and D-forms) and nonracemic (the remaining fraction) aspartic acids. It may be found algebraically that the racemic fraction is equal to twice the difference between the total and L-aspartic acids and that the nonracemic fraction equals the difference between the total and twice the L-aspartic acid.
3. This investigation (No. 115) was aided by grants from the Eli Lilly Company, the Nutrition Foundation, Swift and Company, the National Multiple Sclerosis Society, and the University of California. We are indebted to Evelyn Brown and Arthur Yuwiler for technical assistance.
4. Prepared by L. E. McClure essentially according to the procedure described by Dunn *et al.* [*J. Biol. Chem.* 155, 591 (1944)]. The product contained 14.13 percent nitrogen, 10.19 percent moisture, and 1.66 percent ash.
5. Calculated as percentage of moisture and ash-free material.
6. The differences between 7.21 (the mean total aspartic acid value) and twice the L-aspartic acid values.

27 September 1956

Interaction of Hormonal Effects: Influence of Triiodothyronine on Androgen Metabolism

It has been found (*1*) that triiodothyronine can markedly alter the relative proportion of urinary androsterone and etiocholanolone produced from endogenous or exogenous precursors without any change in the total amount of these two steroid metabolites. In Table 1 the endogenous production of androsterone and etiocholanolone in two patients during a control period without treatment is compared with that found during administration of 200 μg per day of triiodothyronine. It is evident that the thyroid hormone caused an increase in the amount of androsterone with a concomitant fall in the amount of etiocholanolone. Examination of an untreated myxedematous patient showed that etiocholanolone in the urine was 7 times higher than androsterone, despite a low level of production of these metabolites.

With this evidence that elevation of thyroid hormone increased the formation of androsterone, while a diminished thyroid secretion favored the production of etiocholanolone, it was desirable to study the influence of triiodothyronine on the metabolic products produced from

Table 1. Change in proportion of endogenous steroid metabolites with triiodothyronine. Subject P, surgical diagnosis of Stein-Leventhal syndrome; subject F, multiple sclerosis, steroid production and metabolism normal in all respects, from repeated studies in these laboratories. The dose of triiodothyronine was 200 μg per day for a period of 7 (P) and 9 days (F) prior to the studies. The methods used for steroid isolation and analysis have been described (*3*).

Subject	Treatment	Androsterone (mg/24 hr)	Etiocholanolone (mg/24 hr)
P – ♀	Control	3.8	3.9
	T – 3	5.3	2.3
F – ♂	Control	2.2	3.3
	T – 3	3.1	2.7

Table 2. Change in proprotion of metabolites of testosterone-4-C^{14} with triiodothyronine. Subject F, see Table 1; subject M, multiple sclerosis, otherwise in good health.

Subject	Treatment	Androsterone (%)*	Etiocholanolone (%)*
F – ♂	Control	37	44
	T – 3	53	29
M – ♀	Control	21	59
	T – 3	59	29

* Percentage recovered of the total radioactivity in the neutral steroid fraction of the urine, first 24 hours after hormone injection, after hydrolysis of conjugates with β-glucuronidase (Ketodase). The amount of each steroid present was measured by addition of weighed amounts of carrier, purification to radiochemical homogeneity, and calculation from the total radioactivity present in the extract and in the pure steroid. Details of isolation, purification, and measurement were essentially similar to those previously described (*2*).

testosterone-4-C^{14}. It is well known that androsterone and etiocholanolone are the major and nearly exclusive end products of this hormone (*2*).

Tracer amounts of radioactive testosterone were given intravenously in saline solution over a period of 30 minutes to a male and a female subject during a control pretreatment period and 10 days later while they were under treatment with 200 μg per day of triiodothyronine. The results, shown in Table 2, demonstrate that the metabolism of exogenous hormone was altered in the same direction as that observed with endogenous precursors. This effect, similarly, was achieved without any essential change in the total amount of the two metabolites recovered from the testosterone injected.

It can be concluded from these experiments that a thyroid hormone markedly influences the metabolism of androgen from endocrine glands as well as that artificially introduced into the body. The significance of these observations, as well as the details of the study and its extensions, will be described in subsequent reports (*4*).

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References and Notes

1. This investigation was supported by a grant from the American Cancer Society, and by a research grant (C-440) from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service. The initial observation with subject P was made in collaboration with Rulon W. Rawson, division of clinical investigation, Sloan-Kettering Institute, to whom we express our thanks for his cooperation.
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10 October 1956

Formation of UDPGla and Related Compounds by the Soluble Fraction of Liver

In a recent publication (*1*), it was reported that, although an extract of rat liver nuclei formed UDPG (*2*) and UDPGla from UTP and glucose-1-phosphate and glucosamine-1-phosphate, respectively, the formation of UDPAG from UTP and N-acetyl glucosamine-1-phosphate could not be demonstrated. This result was unexpected, since the pyrophosphorolysis of UDPAG by rat liver nuclei described by Smith and Mills (*3*) should be reversible. It thus seems likely that UDPG and UDPAG are synthesized by separate enzymes and that our preparations of nuclear extract were devoid of the second enzyme.

Studies were, therefore, conducted to determine whether the system that forms UDPAG was present in other fractions of the cell (*4*). The best results were obtained with the supernatant fraction of rat-liver homogenate. The protein-free reaction mixtures were chromatographed by a gradient-elution method similar to that described by Hurlbert *et al.* (*5*), and the nucleotides were determined by their light absorption at appropriate wavelengths.

Preliminary studies indicated that when UTP had been incubated in the presence of the soluble fraction of rat liver, a peak corresponding to UDPG appeared. This probably resulted from the breakdown of glycogen to glucose-1-phosphate, which then reacted with UTP to form UDPG. When synthetic N-acetyl glucosamine-1-phosphate (*1*) was added to the supernatant in addition to UTP, the UDPG peak appeared to overlap with that of another uridine-containing nucleotide. These combined peaks were lyophilized and hydrolyzed in 0.01*N* HCl for 30 minutes. The presence of N-acetyl glucosamine in the hydrolyzate was revealed by the method of Reissig *et al.* (*6*). Glucose was shown to be present by a micro method involving the use of glucose oxidase, which does not react with either glucosamine or N-acetyl glucosamine.

Better separation of the nucleotide components was achieved with the solvent combinations described in Fig. 1. A control or zero time sample has been omitted, for the nucleotides in the supernatant fraction of rat liver and in the UTP were found to be negligible in quantity. The contention that glucose-1-phosphate was present in the liver supernatant fraction or resulted from glycogen breakdown is supported by the enhanced formation of UDPG upon the addition of glucose-1-phosphate (compare *A* and *B*, Fig. 1). The higher concentration of UMP in *B* as compared with that in the other experiments is explained by the finding that the liver fraction rapidly breaks down UDPG to uridine, UMP, and UDP.

The synthesis of UDPAG from UTP and N-acetyl glucosamine-1-phosphate is shown in Fig. 1*C*. UTP is probably the primary nucleotide in the conversion, for, though UDP was found to support the synthesis of UDPAG, it was less effective than UTP. UDP is probably active only as a result of its conversion to UTP by a myokinase-type reaction (*7*). A transferase reaction between N-acetyl glucosamine-1-phosphate and UDPG has not been entirely eliminated, but so far we have been unable to obtain evidence for the existence of this reaction. Fig. 1*D* reveals the formation of UDPGla from UTP and glucosamine-1-phosphate as described previously for rat

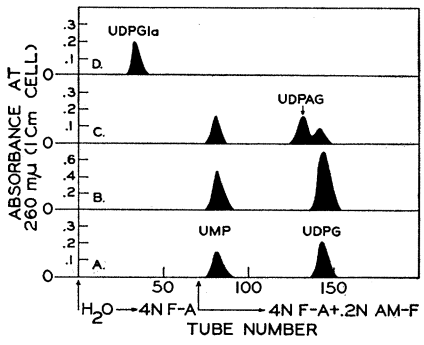

Fig. 1. The reaction mixture in each experiment contained 1 ml of a 20-percent rat-liver homogenate prepared in 0.15*M* KCl and centrifuged (20,000*g*) for 30 minutes to remove the particulate components; 0.08*M* tris-hydroxymethylaminomethane buffer at *p*H 7.6; 0.01*M* KF; 0.006*M* $MgCl_2$; and 0.008*M* UTP. The added substrates were as follows: (*A*) none; (*B*) 0.008*M* glucose-1-phosphate; (*C*) 0.008*M* N-acetyl glucosamine-1-phosphate; and (*D*) 0.008*M* glucosamine-1-phosphate. The total volume in each case was 2.5 ml, and the incubation time was 15 minutes at 30°C. The reaction was stopped by the addition of 1 ml of 10-percent trichloroacetic acid. The protein was removed by centrifuging and, after neutralization, the soluble portion was placed on a 20- by 1.2-cm Dowex-1 formate column. The mixing chamber contained 250 ml of H_2O initially, and 5-ml fractions were collected in each tube.

liver nuclei (*1*). The chromatogram in this experiment was not extended farther than the elution of the UDPGla peak. This nucleotide has not yet been found as a naturally occurring physiological substance, but its possible occurrence cannot be ignored.

Our finding that the conversion of UTP to UDPG is much more rapid than is conversion to UDPAG is consistent with the finding of Brumm, Siekevitz and Potter (*8*) that liver homogenate incorporates phosphorus-32 into UDPG more rapidly than into UDPAG.

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