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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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 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.

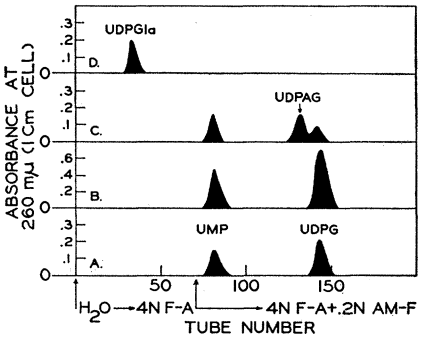

Fig. 1. The reaction mixture in each experiment contained 1 ml of a 20-percent rat-liver homogenate prepared in 0.15*M* KCl and centrifuged (20,000*g*) for 30 minutes to remove the particulate components; 0.08*M* tris-hydroxymethylaminomethane buffer at *p*H 7.6; 0.01*M* KF; 0.006*M* $MgCl_2$; and 0.008*M* 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.

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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References and Notes

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Effects of Fluorocompounds on Mechanisms Related to Mitochondrial Phosphate Transfer

While studying oxidative phosphorylation in rat liver mitochondria (*1*), we have found that the P/O ratio of such preparations is markedly lowered on addition of fluoroacetate to the system (Table 1) (*2*). The effect of this compound on the adenosine triphosphate (ATP) that is formed can be seen before any appreciable inhibition of oxygen consumption is observed, supporting the belief that processes regulating phosphate transfer have a higher sensitivity to the fluorocompound than do the respiratory steps associated with the phosphorylation. In view of these findings, the conclusion (*3*) that fluoroacetate has no effect on oxidative phosphorylation cannot be supported.

Since other experiments showed that the hexokinase used to "trap" the ATP was not affected by fluoroacetate, fluorocitrate, or fluoropyruvate, the lowered P/O ratio could be due to the effect of the fluorocompound on the synthesis of ATP or to an accelerated dephosphorylation of the ATP after it was formed. Attempts to show an effect on the synthesis of ATP by using the mitochondrial extract described by Lehninger (*4*) have been complicated by the attendant ATPase activity of the preparations, although it was shown that fluoroacetate decreases the net incorporation of P^{32} into ATP in the reaction

$$ADP + P^{32} \rightarrow ATP^{32}$$

In vivo experiments (*5*) have shown a small but significant decrease in labile nucleotide phosphorus in the brain following injection of fluoroacetate, and on comparing the effects of fluoroacetate, fluorocitrate, fluoropyruvate, and 2–4 dinitrophenol (DNP) on the release of phosphate from adenosine monophosphate (AMP), adenosine diphosphate (ADP), and ATP by liver mitochondria, we observed (Table 2) that the dephosphorylation of all three nucleotides was stimulated. Fluorocitrate was by far the most active of the fluorocompounds, showing stimulation even at $10^{-6}M$, while fluoroacetate was only slightly active. Interestingly, DNP and the fluorocompounds at very low concentrations produced consistently lower hydrolysis of the nucleotides than did the controls. It is of significance that, whereas the greatest stimulation of phosphate release by the fluorocompounds is seen when AMP is the substrate, the stimulation of dephosphorylation produced by DNP is greatest when ATP is used. Since the mitochondria are capable of rapid interconversion of these three nucleotides, as demonstrated chromatographically, the reactions undergone by a given nucleotide are numerous, and studies on the time-courses of these interconversions in the presence and absence of the fluorocompounds are currently under investigation in an attempt to identify the site(s) of action of these compounds.

That a certain degree of structural integrity of the mitochondria is necessary for these effects is demonstrated by experiments in which the mitochondria have been "pre-aged" by the method of Lardy (*6*): under these conditions, the ability of the fluorocompounds and of DNP to stimulate the dephosphorylation of the nucleotides is greatly diminished. Hunter (*7*) has shown that DNP has no action on the partially purified ATPase of potato, and we have now demonstrated that the soluble 5′-nucleotidase of rattlesnake venom (*8*) is affected by neither DNP nor fluoroacetate nor fluorocitrate.

It does not appear that the fluorocompounds exert their effects by combining with some metal within the mitochondria, for the addition of equivalent concentrations of Versene has no effect on the dephosphorylating activity of the mitochondria. Furthermore, it was found that oxalic acid ($3.7 \times 10^{-5}M$) suppresses mitochondrial ATPase.

We have found that *p*-chloromercuribenzoate (PCMB) and iodoacetate stimulate dephosphorylation of these nucleotides, iodoacetate being much less active than fluoropyruvate. This finding is of particular interest now, in view of the recently demonstrated interaction between fluoropyruvate and sulfhydryl compounds in a model system (*9*).

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Table 1. Effect of fluoroacetate on oxidative phosphorylation. The flasks contained the following: $0.02M$ tris buffer at *p*H 7.28; 6 μmole of $MgCl_2$; 120 μmole of KCl; 20 μmole of potassium fumarate; 25 μmole of KF; 0.5 μmole of ATP; 40 μmole of potassium phosphate; 1 ml of rat liver mitochondria in $0.25M$ sucrose; 744 μmole of sucrose; 30 μmole of glucose; and 0.5 mg of hexokinase added from a side arm, with a final volume of 3 ml. The contents were incubated for 15 minutes at 37°C in oxygen. The reaction was stopped by 0.5 ml of 20-percent trichloroacetic acid.

Item	Oxygen (μatoms)	Phosphate (μmole)	P/O
Control	2.58	6.39	2.48
Fluoroacetate ($3.3 \times 10^{-3}M$)	2.55	3.81	1.49

Table 2. Comparative effects on nucleotide hydrolysis. The flasks contained the following: 10 μmole of nucleotide freshly neutralized to *p*H 7.28; 744 μmole of sucrose; 6 μmole of $MgCl_2$; 120 μmole of KCl; $0.02M$ tris buffer at *p*H 7.28; and mitochondria (from approximately 300 mg of rat liver) in $0.25M$ sucrose. The final volume was 3 ml. The contents were incubated for 20 minutes at 30°C in a Dubnoff shaking incubator. Phosphate was determined by the method of Gomori (*10*). The values given have been corrected for endogenous phosphate. The figures in parentheses indicate percentage stimulation above the respective controls.

System	Phosphate (μmole) released from		
	AMP	ADP	ATP
Control	1.59	3.31	5.85
DNP ($10^{-5}M$)	2.37 (48%)	7.91 (138%)	14.16 (142%)
Control	1.19	3.76	5.99
Fluoroacetate ($10^{-3}M$)	0.97 (−19%)	4.34 (15%)	6.05 (0)
Control	0.88	2.22	4.31
Fluorocitrate ($1.3 \times 10^{-5}M$)	1.77 (100%)	3.80 (70%)	6.34 (47%)
Control	0.71	1.65	4.40
Fluoropyruvate ($10^{-4}M$)	1.31 (84%)	2.94 (73%)	5.60 (27%)
Control	1.48	3.28	5.94
Iodoacetate ($10^{-4}M$)	2.00 (35%)	4.14 (26%)	6.56 (5%)
PCMB ($10^{-4}M$)	2.57 (73%)	5.71 (74%)	9.28 (56%)

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