control levels of 100 to 130 mm-Hg by constricting the aorta above the renal arteries. Total renal blood flow remained essentially unchanged with these small decreases in mean perfusion pressure to levels still within the physiologic range. Oxygen saturation of renal-vein blood samples did not alter from control values, being more than 85-percent saturated throughout all experiments.

The first significant change in the character of the arterial perfusion of the kidneys that follows partial constriction of the aorta is a sharp drop in pulse pressure and pulsatile flow accompanied by little, if any, change in mean pressure and mean flow; this alone did not cause increased amounts of pressor material to appear in renal-vein blood. Further constriction by the band around the aorta caused a further decrease in pulse pressure and pulsatile flow and then it began to lower mean pressure but without change in mean flow. A point is reached between 80 to 95 mm-Hg when increased pressor material is detectable. Figures 1 and 2 show the effect of lowering renal-perfusion pressure in stages in one typical experiment. In Fig. 1 a sharp drop in pulse pressure and pulsatile flow with little change in mean pressure or mean flow was not accompanied by any increase in pressor activity in renal-vein plasma. On reducing mean perfusion pressure to 90 mm-Hg without change in mean blood flow (Fig. 2), considerable pressor material appeared in renal-vein plasma and lesser amounts in arterial plasma. As in the previous experiments, chemical and pharmacologic tests indicated that the material in incubated plasma is angiotensin.

It thus appears that release of renin from the kidney involves a baroceptor, but one not sensitive to reduction of pulse pressure alone. Whether superimposing a normal pulse pressure on a reduced mean perfusion pressure will cause cessation of release is not known, but this may occur if renal baroceptors resemble those in the carotid sinus which are more effectively stimulated by a pulsatile than by a steady pressure (*9*).

Since small reductions in renal perfusion pressure to levels still within a physiologic range, but not causing renal ischemia, are sufficient to cause release of renin, the renin-angiotensin system may be a physiologic mechanism that functions in the normal control of arterial pressure. In addition, since angiotensin has been implicated as the trophic hormone for aldosterone secretion, the average pressure level at the renal baroceptor could have an important influence on normal salt and water metabolism as has been suggested by Tobian *et al.* (*10*). Rather than the entirely pathologic system it has generally been suspected of being, it may in the future have to be considered as another physiologic mechanism that, by becoming unregulated, can cause disease (*11*).

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Thyroid Hormones: Control of Terminal Oxidation

Abstract. *Triiodothyronine administered to thyroidectomized rats preferentially increased the total capacity of the electron transport phosphorylation system when isolated mitochondria from the liver were tested with different substrates, but it caused little increase in the activity of the slowest dehydrogenases and no uncoupling. The increased activity appeared to be partly due to direct activation of some component of the electron transport system, although triiodothyronine injection also stimulated the incorporation of amino acids by isolated mitochondria.*

Since thyroxine can uncouple oxidative phosphorylation in vitro (*1*), many investigators believe that the thyroid hormones control terminal oxidation by regulating the efficiency of energy conservation, possibly by inducing mitochondrial swelling (*2*). However, recent work (*3, 4, 5*) has raised considerable doubt about there being any connection between mitochondrial swelling or uncoupling and the influence of physiological levels of the thyroid hormones on the rate of oxygen uptake.

Tata *et al.* (*5*) have suggested two alternative ways in which the thyroid hormones might influence oxygen consumption. They considered it most probable that some process which required energy was stimulated by the hormones, and observed that when triiodothyronine was administered to thyroidectomized rats, there was an increase in the rate of incorporation of amino acids into microsomal protein before the rate of glutamate oxidation was stimulated. However, the results of their experiments also suggested that triiodothyronine increased the electron transport capacity of the mitochondria more markedly than it increased the activity of any of the individual oxidative steps. It is the purpose of this paper to provide evidence that changes in the level of the thyroid hormones affect preferentially the total capacity of the electron transport system without affecting the efficiency of phosphorylation.

Male Wistar rats were thyroidectomized surgically and kept for 5 to 8 weeks prior to use. They were used only if they showed steady weight gains of 0 to 6 g per week for at least 2 weeks (*6*). Suspensions of mitochondria were prepared from liver (*7*) and oxygen consumption was measured with the Clark oxygen electrode (*8*). The suspensions were each incubated with various substrates, and during each incubation period the rate of oxidation of the substrate in the presence and absence of 1 μmole of ADP (*9*), was measured, and estimates of the ratio of P to O (*9*) and the respiratory control ratio (*6*) were obtained.

The results of the experiments are shown in Table 1. Although they are similar to those reported by Tata *et al.* (*5*), they also show that the rate of oxidation of the substrates which have NAD-linked dehydrogenases was affected more markedly when they were mixed together than when they were tested individually. Furthermore, there

was little change in the rate of oxidation of those substrates which were oxidized most slowly: malate, pyruvate, and citrate. These data indicate that the observed changes in dehydrogenase activity could have little effect on the overall rate of the citric acid cycle. Nevertheless, the rates of oxidation of the mixture of substrates and of succinate show that changes in the level of thyroid hormones produced great changes in the capacity for oxidative phosphorylation. Although the data are not included here, neither thyroidectomy nor injection of the rats with triiodothyronine significantly changed the P to O ratios, or the respiratory control ratios, which were obtained with any of the substrates or with the mixture of substrates.

As reported previously (*6*) thyroxine, added in vitro, stimulated the oxidation of succinate by mitochondria from thyroidectomized rats. A similar study of other substrates has demonstrated that $2.5 \times 10^{-5} M$ thyroxine inhibited the oxidation of all of the substrates which have NAD-linked dehydrogenases by 5 to 20 percent. Despite these inhibitory effects, thyroxine stimulated the oxidation of the mixture of six substrates by approximately 15 percent. Neither the P to O ratios nor the respiratory control ratios were reduced by the addition of thyroxine in vitro. These results show that thyroxine can produce a direct stimulation both of electron transport and of the associated phosphorylation reactions when added to mitochondria from thyroidectomized rats. No increases in the rate of oxidation of succinate or of the mixture of substrates were obtained when thyroxine was added to normal mitochondria or to those from thyroidectomized rats injected with triiodothyronine.

The effects of thyroxine, added in vitro, on the rate of oxidation were small compared to the stimulatory effects of triiodothyronine shown in Table 1. When triiodothyronine was tested in vitro it produced effects similar to those obtained with thyroxine. To test the possibility that the difference between the effects of the thyroid hormones in vitro and in vivo might be due to an increased rate of synthesis of mitochondrial components in vivo, we investigated the incorporation of an amino acid by isolated liver mitochondria. The general procedure described by Truman and Korner (*11*) was used, with some modifications of the incubation medium. No cell-sap or other supernatant fraction was added. The results are shown in Table 2. For each batch of mitochondria, the rate of incorporation of leucine-1-C^{14} into the total mitochondrial protein was measured and found to be approximately linear up to 1 hour. In addition, a portion of each mitochondrial suspension was incubated with leucine-1-C^{14} for 1 hour and then used to prepare phosphorylating submitochondrial particles by the method of Kielley and Bronk (*8*). Suspensions of protein from the submitochondrial particles, as well as the remaining supernatant protein, were prepared for counting by the same procedure that was used for the total mitochondrial protein (*11*). It is evident from Table 2 that the rate of incorporation of leucine-1-C^{14} was reduced by thyroidectomy and elevated by triiodothyronine injection, and that in all three instances a disproportionately large amount of the labeled amino acid appeared in protein closely associated with the oxidative phosphorylation enzyme system.

The results reported here indicate that the thyroid hormones are capable of producing rapid increases in the terminal oxidative capacity of liver mitochondria, both in vivo and in vitro, without causing comparable increases in the activities of the individual dehydrogenases. Furthermore, since the increases in oxidative capacity were not associated with a loss of phosphorylation efficiency, they must be accompanied by similar increases in the activity of the enzymes associated with phosphorylation. Although the stimulation of oxidation by the thyroid hormones in vitro is clearly an effect of activation, the larger effects which occur in vivo may be due, at least in part, to an increased synthesis of some components of the electron transport chain or its associated enzymes. This view is supported by the fact that the rate of incorporation of the amino acid by isolated mitochondria was much more

Table 1. The influence of changes in the level of thyroid hormones in vivo on the rate of oxidation of substrates by isolated mitochondria. Conditions: 200 μmoles sucrose; 20 μmoles phosphate, *p*H 7.0; 10 μmoles $MgCl_2$; 10 μmoles of each substrate except 20 μmoles of β-hydroxybutyrate. The mixture of substrates which have NAD-linked dehydrogenases contained 2 μmoles each of α-ketoglutarate, malate, pyruvate, citrate, glutamate, and β-hydroxybutyrate; 1 μmole of ADP added 2 minutes after approximately 0.5 mg of mitochondrial nitrogen; 28°C; 2 ml total volume. Each value is given as the mean of duplicate observations with four animals ± the standard error of the mean. The injected animals received 30 μg of 3,5,3′-triiodo-L-tyronine subcutaneously 48 hours prior to the experiment.

Substrate	Oxygen consumption (10^{-6} g atoms/min per mg nitrogen)			C/B × 100
	Normal rats	Thyroidectomized rats	Thyroidectomized rats injected with triiodothyronine	
Succinate	1.209 ± 0.041	0.506 ± 0.026	1.184 ± 0.030	234
α-Ketoglutarate	0.693 ± .037	.375 ± .019	0.605 ± .048	161
Malate	.147 ± .006	.155 ± .005	.184 ± .013	119
Pyruvate	.319 ± .017	.187 ± .014	.259 ± .030	139
Citrate	.301 ± .027	.267 ± .018	.370 ± .024	139
Glutamate	.622 ± .028	.370 ± .027	.567 ± .048	153
β-Hydroxybutyrate	.583 ± .024	.363 ± .014	.479 ± .051	132
Pyruvate + malate	.475 ± .013	.349 ± .016	.434 ± .007	124
Mixture of NAD-linked substrates	.957 ± .028	.464 ± .033	.878 ± .057	189

Table 2. The influence of changes in the level of thyroid hormones on the rate of incorporation of leucine-1-C^{14} by isolated mitochondria. Conditions: 110 m*M* sucrose; 25 m*M* tris, *p*H 7.4; 10 m*M* phosphate, *p*H 7.4; 50 m*M* KCl; 10 m*M* nicotinamide; 18 m*M* NAD; 10 m*M* $MgCl_2$; 1 mg/ml of an amino acid mixture; 10 m*M* succinate; 0.5 μc/ml of DL-leucine-1-C^{14} (specific activity 4 mc/m*M*); 37°C. Each sample of total mitochondrial protein contained about 5 mg of protein. Corresponding figures for the protein from the submitochondrial particles and for the supernatant protein were 2 mg and 6 mg, respectively.

Animals	Incorporation of leucine-1-C^{14} (count/min per mg of protein per hr)		
	Total protein	Protein from submitochondrial particles	Supernatant protein
Normal rats	36.5	132.9	12.2
Thyroidectomized rats	8.0	33.2	4.2
Thyroidectomized rats injected with 30 μg triiodothyronine	33.1	148.3	13.7

markedly affected by changes in the level of thyroid hormones than was the rate of incorporation into microsomal protein (*4, 5, 12,* and Table 2).

The stimulation of terminal oxidation by the thyroid hormones could not in itself account for the increase in metabolic rate which follows the injection of triiodothyronine into thyrodectomized rats (*4, 5*). However, since thyroidectomy markedly reduced the growth rate, there are probably many factors which would tend to increase the demand for energy, and hence the rate of oxygen uptake, once the capacity for oxidative phosphorylation was increased by the thyroid hormones. The suggestion made by Tata *et al.* (*5*) that changes in oxidative capacity may simply reflect an adaptation to an increased demand for energy is unlikely, since, in the same study, they also observed that the increases in oxidative activity in the liver preceded the rise in basal metabolic rate. The direct activation effects of thyroxine which have been reported earlier (*6, 13*), and in this paper, also indicate that this adaptive response is improbable. Furthermore, other recent work (*14*) has shown that an increased demand for energy results in a large increase in the activity of the slowest steps of the citric acid cycle with little effect on electron transport capacity. Thus, increased demand for energy produces effects just the opposite of those produced by triiodothyronine.

The stimulation of the electron transport phosphorylation system is probably only one of many effects of the thyroid hormones on metabolism. However, such an action may be an essential prelude to any stimulation of a process requiring energy, such as protein synthesis (*12*). Clearly, any rise in the metabolic rate can only result from an increase in the rate of utilization of energy (*15*).

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Tolerance between Host and Donor Tissue in Birds

Abstract. *Two methods were used to develop tolerance in the host toward donor cells. In the first method chicken embryos were joined by the parabiotic union of the chorioallantoic membranes after 9 days of incubation. The second method consisted of the use of embryonic transplants of limb buds from donor embryo to host embryo. These transplants were made after 96 hours of incubation with chicken embryos. Other species used were incubated to comparable development. Both methods were successful in the development of tolerance. However, the degree of tolerance attained varied within each method.*

One of the limiting barriers faced by skilled surgeons today is the fact that, in general, tissue transplants from one human to another eventually disintegrate and slough away. Certain exceptions to this general rule exist. Cartilage and tissue from the cornea of the eye can be transplanted from donor to host and are both tolerated and nourished. Transplants between members of a pair of identical twins usually give successful "takes."

Beyond these exceptions it appears that tolerance between host and donor tissue must be developed in some manner before transplants develop into successful takes and as such can be retained by the host. Our work with birds has been concerned with the development of this tolerance to a point where the host will accept, tolerate, and nourish the tissue of the donor. To explain the necessity for such a conditioning process, let us examine the situation which develops when tissue transplants are made between unrelated animals which have undergone no conditioning.

Every animal carries certain genes called histocompatibility genes. These have the power to direct the formation of certain structures on the surface of cells called antigens. For each histocompatibility gene a specific antigen develops. If these antigens are transferred on cells to a host animal whose cells do not already carry the same antigen, they very shortly stimulate the antibody system of the host to produce antibodies. These antibodies function in neutralizing the foreign antigens. Once this is fully accomplished, the function of the donor cells becomes impaired and they start to disintegrate. In a short time the transplanted donor tissue is completely eliminated.

Aside from the use of drugs or x-rays, which temporarily inhibit the action of the antibody system, there seems to be only one means by which the antibody barrier can be bridged. In the embryonic and neonatal animal the antibody system is very passive. Some serologists suggest that this is the period during which the antibody system becomes acquainted with the individual's own antigens and accepts them as its own. As a result they will not be attacked by the antibody system when it becomes more active. At any rate we do know that if foreign antigens on donor cells are introduced into the organization of the host during the passive state of the antibody system, it will at times accept them as the host's own antigens. Sometimes this tolerance is lasting; sometimes it is relatively short-lived.

It is well known that the fetal membranes of dizygotic cattle twins (two-egg twins) will often become anastomosed, and the embryonic blood streams of the twins will mix. It has been shown (*1*) that each twin retains for a long time blood cells whose origin traces to the other twin. Furthermore, after birth such twins will accept skin from each other (*2*). This is in contrast to full siblings of separate birth, which normally reject each other's skin.

In the first phase of the work involved in the development of tolerance between host and donor tissue we employed a method (*3*) which artificially creates a situation in birds analogous to that found in dizygotic cattle twins. After 9 days of incubation, windows are cut in the shells of two eggs, expos-