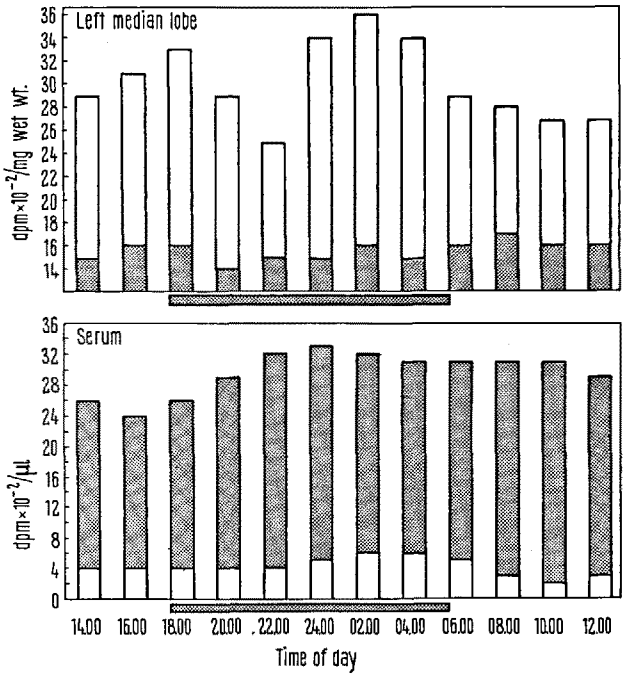
As expected, the TCA-T insoluble radioactivity in the liver began to decrease in the dark period. Then surprisingly, it rose to a high value at 14.00 h and then reversed by decreasing to amounts nearly equal to those seen during the light period, prior to food removal. Hepatic soluble radioactivity was in phase with the insoluble values, except after the lights went back on, when it seemed to exhibit a slight reciprocal relationship. Soluble radioactivity in serum was considerably out of phase with the hepatic insoluble fraction, while serum insoluble radioactivity was generally in phase with that of liver.

The increase in incorporation of ^{3}H-leucine into hepatic protein seen here cannot simply be explained as the result of an increased specific radioactivity of the intracellular leucine pool. If this were so, the TCA-T insoluble radioactivity should have continued to increase almost indefinitely, instead of abruptly decreasing as it did at 04.00 h. In addition, it is well known that essential amino acids such as leucine are conserved during periods of starvation,



Radioactivity in rat serum and liver during a 24-h period. Values are 20 min after ^{3}H-leucine injection. Open block is the trichloroacetic acid-sodium tungstate (TCA-T) insoluble fraction, solid block is the TCA-T soluble fraction. Radioactivity was estimated by liquid scintillation spectrometry. Dimensions of the abcissa for the left median lobe start above the baseline to conserve space. Lights were off from 18.00 h to 06.00 h.

which would have a diluting effect on radioactivity. Such a diluting effect was not observed here. Even so, this interpretation is subject to revision since we do not have data on the actual specific radioactivity of the hepatic intracellular leucine pool.

The slight increase in insoluble hepatic radioactivity seen at the end of the light phase and continuing early in the dark phase could be a reflection of the synthesis of proteins such as tyrosine transaminase, which is highest in activity at 20.00 h[2]. If so, and with respect to the second and largest rise in radioactivity of protein seen here, an uncoupling of biphasic or even multiphasic hepatic protein synthesis as a result of food deprivation is indicated. In other words, feeding might entrain different cyclic aspects of hepatic protein synthesis, with the result that one may appear to act as a primary synchronizer for the other. In fact, if the stressful effects of starvation were not so adversely directed towards protein synthesis[2], it would be interesting to see whether hepatic protein synthesis would as indicated above, continue to be biphasic with something other than a 24 h cycle. Under these conditions, liver protein synthesis would truly be 'circadian' in nature and the ingestion of food could be considered the primary synchronizer or 'Zeitgeber'.

Obviously other interrelated factors such as uncoupling of primary and secondary synchronizers[12], endocrine effects[13], and variable half-life of mRNA species[14] are concerned with this phenomenon. In any case, the fact that a cyclic incorporation of ^{3}H-leucine into hepatic protein still occurs in fasted animals does suggest that general hepatic protein synthesis may be subject to the influence of the 'Biological Clock'[15].

Zusammenfassung. Die Aufnahme von ^{3}H-Leucin in das Lebereiweiss wurde während der Dunkelphase ihres Tag-Nachtzyklus bei fastenden Ratten untersucht. Die übliche rhythmische Aufnahme von ^{3}H-Leucin wurde durch die Fastenperiode nicht verhindert, obwohl die Menge des aufgenommenen ^{3}H-Leucin zwei zeitlich verschiedene Maxima aufwies.

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Angiotensin Tachyphylaxis and Vascular Angiotensinase Activity

It has long been known that smooth muscle preparations frequently become refractory to repetitive stimulation[1]. When the loss of responsiveness is nonspecific, in the sense that responses are lost to all stimuli, it seems likely that this reflects an abnormality of either the contractile process itself or in the metabolic pathways which support contraction. More puzzling is a phenomenon which has been called specific desensitization, or tachyphylaxis[1]. In this situation the contractile process is intact, and the loss of response involves only the agent to which the tissue has been exposed. While this poorly understood phenomenon has been described for a large number of agents and systems, much of the recent interest has focused on angiotensin tachyphylaxis, presumably because of the potential physiological importance of reactivity of the renal vasculature to angiotensin[2,3]

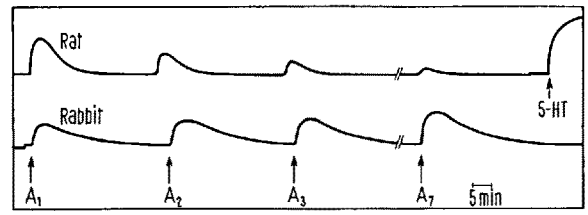
and the wide interest in variations in angiotensin responsiveness in patients with hypertension[4,5]. KHAIRALLAH et al.[6] suggested, on the basis of several observations, that angiotensin tachyphylaxis represents saturation of receptor sites which are normally freed of angiotensin by local angiotensinases, an interpretation which depends on PATON's[7] hypothesis concerning receptor activation. This study deals with the relationships between tissue angiotensinase and propensity to develop angiotensin tachyphylaxis in a number of isolated tissue preparations.

The tissues utilized in this study included the rat, rabbit, and guinea-pig aorta, the rat stomach and colon, and guinea-pig ileum. Aortic strips were cut and mounted according to the method of FURCHGOTT and BHADRAKOM[8] with 4 g tension on rabbit aortic strips, and 1 g on aortic strips from the rat and guinea-pig. The rat fundus and colon were prepared according to the descriptions of VANE[9,10]. Strips were mounted in muscle chambers with a 10 ml working volume (Phipps and Bird) containing a modified Krebs-bicarbonate medium[10], maintained at 37 ± 0.5 °C and constantly aerated with a gas mixture containing 95% O_2-5% CO_2. Isotonic contractions were monitored with a Harvard Instrument force transducer (No. 356), amplifier and galvanometer (No. 350). Approximately the central 4 cm of pen excursion were utilized, recordings being made on a chart-mover (No. 860, Harvard Instruments) at 0.005 cm/sec. An equilibration period of approximately 1 h was allowed before initiating drug administration. The bath arrangement allowed continuous washout for removal of solutions and drugs at appropriate intervals. The volume used for the wash was at least 10 times the bath volume and relaxation of the smooth muscle preparations after removal of the drugs was entirely passive.

Attempts to induce tachyphylaxis to angiotensin (Hypertensin Ciba®) were carried out in two ways. In one group of experiments the tissues were exposed repeatedly to a dose of 10^{-7} g/ml at 30 to 60 min intervals, with washes between each exposure. The second approach involved a prolonged, continued exposure of the tissues to 10^{-5} g/ml without rinsing for 60 to 90 min. Tachyphylaxis was demonstrated by repeating the same dose and the specificity was assessed by challenging the tissue with appropriate agents, usually serotonin or norepinephrine.

Tissue angiotensinase levels were determined in the aortae of all 3 species and the rat stomach. Tissues were minced and placed in an Erlenmeyer flask containing angiotensin at 10^{-5} g/ml in 5 ml Krebs-bicarbonate solution. Incubation was carried out at 37 °C with continuous mixing. In each experiment a control flask containing the solution and angiotensin but no tissue was also incubated. Samples for assay of residual angiotensin were taken from each flask at 5 or 10 min intervals beginning at 1 min for at least 1 h, or until the residual concentration was less than 3% of the peak. Assays were carried out on guinea-pig ileum or rat colon strips. The results presented are for the studies with guinea-pig ileum, which in our hands was the better assay system because of a more stable baseline, less spontaneous activity and a more rapid recovery time after each sample. Dose-response curves were very reproducible. Individual samples were assayed in replicate up to 4 times without a significant effect on calculated angiotensin disappearance. The latter has been expressed as the K value derived from a semilogarithmic plot of angiotensin disappearance, fitted by eye. At least 7 points were utilized for each fit and at least 5 different tissue samples assessed in each group. Spontaneous angiotensin disappearance did not occur in this system in the absence of tissue. Differences between the mean values for each tissue were assessed with the Student t-test.

A clearcut separation was found between the tendency of various tissues to develop angiotensin tachyphylaxis. The rat aorta developed specific angiotensin tachyphylaxis readily, with both repetitive stimulation or a continued exposure, as reported earlier[6]. An example is shown in the Figure. In this preparation, failure to develop angiotensin tachyphylaxis was a very unusual event in over 100 studies. The rabbit aorta, on the other hand, was extremely resistant to the development of angiotensin tachyphylaxis (see Figure). Even a small reduction in responsiveness was unusual. The guinea-pig aorta showed an extremely variable pattern. In some guinea-pigs specific tachyphylaxis developed quickly and with low doses; in others even high dose serial administration or continued exposure did not result in tachyphylaxis. This degree of variability was not described by others[6], but apparently only 2 preparations were assessed. The rat stomach was extremely resistant to the development of tachyphylaxis.



Response to serial doses (10^{-7} g/ml) of angiotensin of rat and rabbit aortic strips. Note the progressive loss of responsiveness to angiotensin of the rat aortic strip and the retained response to serotonin.

Tissue angiotensinase levels

	Absolute K	Normalized K
Rat stomach	0.088 ± 0.15 *	0.138 ± 0.032
Rat aorta	0.020 ± 0.002	0.119 ± 0.013
Rabbit aorta	0.017 ± 0.009	0.066 ± 0.029
Guinea-pig aorta	0.011 ± 0.003	0.085 ± 0.019

* Angiotensinase level has been expressed as a K value describing absolute disappearance $\pm$ S.E.M., and as a K value normalized according to tissue weight. Normalized K values show no significant differences, but note that the 2 tissues which do not display tachyphylaxis – the rat stomach and rabbit aorta – represent the extreme values for normalized K. The absolute K for rat stomach was significantly greater than all vascular tissues ($p < 0.01$).

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Tissue angiotensinase activity is shown in the Table. No differences were found between the total tissue angiotensinase activity per unit mass in any of the tissues studied, nor was tissue angiotensinase activity more variable in the guinea-pig than in the other 2 species. The rat stomach displayed a significantly higher rate of angiotensin destruction, exceeding the level in vascular tissues by a factor of 4, in approximate proportion to the larger mass utilized. It has also been suggested that the rate of relaxation of a contracted vascular strip represents a valid index of overall drug inactivation[11]. The development of tachyphylaxis in some preparations makes such an interpretation suspect, but examination of the Figure makes it clear that relaxation of the rat aortic strip after washout of aqueous phase angiotensin was considerably more rapid than that of the rabbit.

A parallel between total tissue angiotensinase levels in a number of vascular tissues and their propensity to develop angiotensin tachyphylaxis could not be demonstrated in this study. Such a parallel is a requisite of the hypothesis elaborated by KHAIRALLAH et al.[6]. The much more rapid degradation of angiotensin by the rat stomach makes it clear that the assay system could detect differences. In addition, the more variable propensity to develop tachyphylaxis of the guinea-pig aorta was not associated with a more variable angiotensinase activity in that tissue. It thus seems unlikely that total tissue angiotensinase is the critical determinant in angiotensin tachyphylaxis. There is also debate concerning the role of plasma angiotensinases in tachyphylaxis in a number of systems[4], but the considerable washing carried out on the tissues prior to study makes it unlikely that they could have influenced the results. If tissue angiotensinase is critical, and attractive evidence supports that concept[6], specificity must be applied either through the differences in the metabolic products of enzymes in various tissues or in the specific distribution of the enzymes in the tissues. The possibility exists that the location of angiotensinases in the tissue, specifically their precise relationship to the receptor site, may be an important factor. It is important to recognize, however, that saturation of receptors with an agonist can only account for a failure of response if PATON's[7] concept of receptor activation as a rate rather than occupation phenomenon provides an adequate description of receptor activation, a matter of continued debate[1,12-14].

Zusammenfassung. Die Auffassung, dass die Gewebsangiotensinase-Aktivität entscheidend sei, ob sich eine Gewebs-Tachiphylaxie gegenüber Angiotensin entwickelt oder nicht, konnte durch Versuche an verschiedenartigen Geweben keine Bestätigung finden.

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The Oxygen Uptake of the Developing Brain in the Rat with Intra-Uterine Growth Retardation

Our previous studies on body composition of the fetus with intra-uterine growth retardation (IUGR) and about somatic development of these animals until adulthood point out the importance of low blood glucose level and decreased total liver glycogen stores in the experimental rats[1-4]. The formation and oxidation of amino acids constitutes a main pathway of glucose metabolism in the brain[5], and it is probable that most of the liver glycogen which is mobilized after birth is utilized by the brain rather than the body as whole. These findings led us to suggest that the glucose requirements for brain of IUGR rats are higher than the amount available by synthesis, and partly explain the hypoglycemia of the stunted rats.

This report deals with oxygen consumption of cerebral cortex of IUGR and control rats during perinatal development.

Methods. Female rats of Sherman strain are mated overnight; they were fed ad libitum on pelleted diet. After birth only 6 newborn are left per litter. The growth retardation was induced in pregnant rats by artery clamping of 1 horn at 17th day after mating. All the IUGR animals had a reduction of weight of more than 15% as compared to controls. This criterion was determined in a previous work[1].

On the 21th day of gestation, the mother was killed by decapitation and the fetuses were extracted immediately by Caesarean section. After the birth, the animals were killed by decapitation and brains were quickly removed, dissected and the cerebral cortex weighed and kept for chemical analyses.

Oxygen consumption was determined by the conventional Warburg technique. Homogenates (10% W:v) were prepared in 'an isotonic NaCl solution' according to ELLIOT et al.[6]. This solution was prepared freshly each day. All manometric experiments were carried out in duplicate at 37 °C. Air was used as the gaseous phase. After 10 min of thermal equilibration, oxygen consumption was measured for 30 min. The results were expressed as μmoles O_2/H/g wet tissue.

At each age, dry weights were obtained by heating the tissue in an oven to a constant weight. Aliquots were used for the subsequent determinations: a) Protein with Folin/Ciacalteu reagent by the LOWRY procedure. b) Des-

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