Significant differences in neurotransmitter uptake at 08.00 h and 20.00 h

Neurotransmitter	Region	Uptake (as median tissue/medium ratio) 08.00 h	20.00 h	Significance[a] (p)
Male rats (n = 14)				
DA	Hypothalamus	17.6	15.8	< 0.05
DA	Striatum	65.2	52.2	< 0.01
NA	Hypothalamus	19.6	17.3	< 0.05
5HT	Hippocampus	16.8	13.8	< 0.05
Ovariectomized female rats (n = 14)				
DA	Cortex	16.0	10.3	< 0.05
DA	Striatum	62.5	52.5	< 0.01
DA	Midbrain	14.5	13.0	< 0.05

Regions investigated: Cortex, hypothalamus, thalamus, midbrain, striatum, hippocampus, pons and medulla. [a] Wilcoxon test for paired values.

5HT and NA maxima are not simple effects of the external temperature, although this is contributory. Seasonal rhythms occur in many vertebrates primarily in relation to endocrine functions. Such a seasonal adaptive capacity might be expected to have an important selective value which is retained even in laboratory animals kept under controlled conditions.

Rhythmic phenomena in the metabolism of the monoamines may have far-reaching consequences. For example, periodicity is a well-established characteristic of manic-depressive illness, whether it be its cyclic nature, whether the circadian mood changes often associated with alterations of sleep-waking patterns and corticosteroid rhythms, or the seasonal increase in depressions in autumn and early spring[19]. Metabolic rhythmicity of the amines thought to be involved in the aetiology of depression[20] may be one of the factors causing periods of increased susceptibility.

Zusammenfassung. Eine signifikante höhere Neurotransmitter-Aufnahme um 08.00 h als um 20.00 h wurde für Dopamin im Striatum und Hypothalamus, Noradrenalin im Hypothalamus, und Serotonin im Hippocampus von männlichen Ratten gefunden, aber keine Veränderung der GABA-Aufnahme. Ähnliche Unterschiede wurden für Dopamin im Cortex, Striatum und Mittelhirn von ovarektomierten Ratten gefunden. Ausserdem wurde eine gegenläufige jahresrhythmische Veränderung der Dopamin- und Serotonin-Aufnahme im Striatum beobachtet.

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Plasma Enzyme Activities in Rats with Diet-Induced Alterations in Liver Enzyme Activities[1]

A variety of intracellular enzymes can be found in minute quantities in the blood[2]. The plasma level of an enzyme clearly depends upon the rate of its loss from tissues and the rate of its clearance from plasma. However, a lack of specific information on this topic and the variety of organs possessing a particular enzyme, make it difficult to estimate the basic determinants of the activity of an enzyme in the plasma.

Table I. Diet composition (% by weight)

	20 P	45 P
Casein	20.0	45.0
Sucrose	63.7	38.7
Corn Oil	10.0	10.0
Salts Mix	3.8	3.8
Vitamin Mix	2.5	2.5
Caloric density	4.11 cal/g	4.11 cal/g

Physical training leads to an increase in the resting level of lactic dehydrogenase (LDH) in the plasma of man[3], and of glutamic-oxalacetic transaminase (GOT) in the plasma, and skeletal and cardiac muscle of rats[4]. The increased resting levels of plasma enzyme activity following training could result possibly from either an increase in the level of their activity in certain tissues, or the repeated loss of tissue enzyme during the exercise comprizing the physical training. This latter mechanism seems unlikely since HUNTER and CRITZ[3] have found that in man the elevation of plasma LDH occurring during maximal exercise was eliminated by physical training. This suggests then, that the amount of tissue activity of an enzyme could be an important determinant of the plasma activity of that enzyme.

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Table II. Body, liver, liver/body weight ratio, and haematocrit [a]

Group	Body weight (g)	Liver weight (g)	Liver/body weight ratio (%)	Haematocrit (%)
20 P	225 ± 3	10.01 ± 0.42	4.02 ± 0.12	40.5 ± 0.7
45 P	235 ± 2 [b, c]	9.08 ± 0.33	4.29 ± 0.17	41.4 ± 0.9

[a] Values are means ± SEM. [b] Significantly different ($p < 0.05$, $p < 0.001$).

ZIMMERMAN et al.[5] observed a good correlation between the tissue and serum levels of GOT and glutamic-pyruvic transaminase (GPT) in 6 species of vertebrates. In particular the liver and serum activities appeared well correlated. In view of the relatively large size of the liver, its high rate of protein turnover, and its high level of enzyme activity, it seemed a reasonable hypothesis that the liver may be a principal source of several serum enzymes.

To test this hypothesis, the plasma and liver activities of 4 enzymes were assayed in 2 groups of rats fed on diets of differing protein content and identical caloric density. The activity of many liver enzymes is influenced to a great extent by the amount of dietary protein, such that several-fold differences in their activity may arise[6].

The enzymes we chose to examine were: 1. GPT, found predominantly in the liver but with some activity present in the kidney, cardiac and skeletal muscle; 2. GOT, with high activity in liver, cardiac muscle, skeletal muscle and erythrocytes; 3. creatine phosphokinase (CPK), which is found mainly in neural and muscular tissue, demonstrating very little activity in other tissues, and 4. LDH, common in cardiac muscle, kidney, skeletal muscle, liver and erythrocytes.

Experimental methods. Male rats of the Sprague Dawley strain, initial weight 149 g, were randomly assigned into 2 groups. After 1 week on a diet of standard laboratory rat chow and water ad libitum, groups were fed diets containing either 20 (20 P), or 45% (45 P) casein (Table I) and allowed both diet and water ad libitum.

Two weeks later, animals were anaesthetized with sodium pentobarbital (35 mg/kg). Blood (5–10 ml) was obtained by intracardiac puncture, heparinized, centrifuged, and the separated plasma refrigerated at 4 °C. The haematocrit was determined. Any haemolyzed samples were discarded.

The liver was quickly removed, weighed, and a small portion (150–200 mg) homogenized (1:100 w/v) in cold 0.1 *M* phosphate buffer (pH 7.4), centrifuged at 10,000 *g* for 10 min, and the supernate stored at 4 °C for later analysis.

Enzyme determinations were carried out on the plasma and the liver supernate. CPK was assayed, within 2 h of obtaining the samples, according to the method of OLIVER[7]. All other enzymes were assayed within 48 h. GOT and GPT were determined according to the methods of KARMEN[8] and WROBLEWSKI and LADUE[9] respectively. Total LDH was assayed following the method of BABSON and PHILLIPS[10].

The enzyme activities of the plasma and liver were expressed in IU/l plasma, and per gram of wet tissue (IU/g), respectively. 8 plasma samples were obtained from each group. All other determinations on each group were made on 11 animals. Comparison between the means of each group was made using the Student *t*-test.

Results and discussion. Rats fed the 45 P diet showed a larger body weight gain (Table II). The liver: body weight ratio was the same for each group, thus changes in enzyme activity per gram of liver reflected similar changes in total liver enzyme activity as related to body weight.

Plasma levels of GOT (PGOT) were higher in 45 P rats (Table III). There was a corresponding difference in liver activity with 45 P rats demonstrating 60% more activity than 20 P. With the higher level of dietary protein, significantly higher plasma levels of GPT (PGPT) were observed (Table III). Compared with 20 P rats the liver activities of GPT in 45 P rats were over 200% greater. There was a wide range of activity of LDH in the plasma (PLDH) of both groups, with no significant difference between groups (Table III). The liver LDH activity was the same in both 45 P and 20 P rats. The PCPK

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Table III. GOT, GPT, LDH, and CPK activity in plasma (IU/l) and liver (IU/g) [a]

	Plasma		Liver	
	20 P	45 P	20 P	45 P
GOT	42.1 ± 2.0	57.0 ± 5.8 [b]	52.1 ± 2.0	86.2 ± 6.5 [b]
GPT	19.2 ± 0.8	23.3 ± 0.8 [b]	108 ± 9	262 ± 20 [c]
LDH	66.0 ± 7.2	54.6 ± 7.1	258 ± 22	209 ± 18
CPK	105 ± 18	110 ± 5	—	—

[a] For superscripts see Table II.

activity was variable with no difference between 20 P and 45 P rats. No CPK activity could be demonstrated in the liver.

The different dietary levels of protein resulted in variations in the liver activities of LDH, GPT and GOT similar to those described by SCHIMKE[6]. In animals on a high protein diet, an increase in the activity of the liver transaminases is part of a biochemical adaptation associated with increased amino acid metabolism. Likewise, the lower liver activity of LDH in these rats probably coincides with a decrease in the utilization of the glycolytic pathway.

Although the high protein diet induced much larger increases in liver GPT than in liver GOT, the differences in PGOT and PGPT were of opposite magnitude. This might be due to the varying cellular locations of the enzymes, or to their different plasma half lives.

There is a possibility that enzyme activities in other tissues were modified by the amount of dietary protein. However, it is likely that the higher levels of PGOT and PGPT in the 45 P rats were related in part, to the higher liver activities of these enzymes.

EGGLESTON and KREBS[11] have found 2 to 3-fold differences between the liver activities of 3 glycolytic enzymes in 4 strains of rats (3 Wistar derived; 1 Sprague Dawley) fed the same diet. Such apparent intrinsic strain differences in enzyme activity, together with variations induced by diet, could result in widely varying activities of many enzymes in both the liver and plasma. We feel, therefore, that considerable caution is warranted in the comparison of data on plasma enzyme activities obtained in different laboratories.

Zusammenfassung. Die Korrelation von Leberenzymen mit entsprechenden Plasmawerten wird diskutiert.

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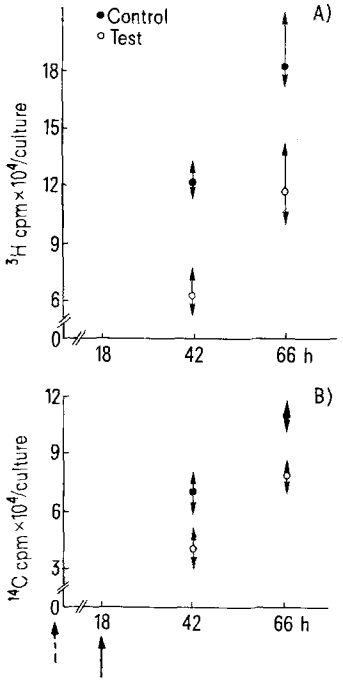
Decreased Synthesis of 'Secreted' Proteins in Chick Embryo Liver Cultures Treated by Estradiol-17-β

A decrease of all normal serum components accompanying a striking increase of liver-produced yolk proteins takes place in the laying hen or in both sexes following treatment with estrogen[1]. Since chick embryo liver primary cultures are able to produce and secrete into surrounding medium both serum proteins[2] and, after estradiol-17-β stimulation, a yolk protein, phosvitin[3], we have examined whether a similar effect can be observed in vitro. We have therefore studied the synthesis of total 'secreted' proteins by the estradiol-17-β treated liver cells after 42 h and 66 h in vitro i.e. during the period of phosvitin synthesis induction[3]. 14-day-old chick embryo liver cultures are performed as previously described[3]. Test cultures are supplied with nutrient containing 500 γ of estradiol-17-β (Merck; 50 γ/μl dissolved in propylene glycol) for 18 h. Media are then replaced and 2 independent sets of experiments are carried out. In the first experiment both control and hormone treated cultures are supplied with the nutrient containing 10 μCi/culture of L-(^{3}H)leucine (New England Nuclear Corporation; specific activity 38.58 Ci/mmole); in the second one with the nutrient containing 1 μCi/culture of L-(^{14}C) serine (New England Nuclear Corporation; specific activity 0.154 Ci/mmole). After collection and centrifugation (50 *g*/10 min) culture media are precipitated by 10% trichloroacetic acid, washed twice with 5% TCA, methanol-ethylic ether (1/1, v/v) and ether. Precipitates are then dissolved in about 1 ml of a protein solubilizer (soluene, Packard Instrument Co. Inc.) and mixed with 10 ml of spectrafluor PPO-POPOP scintillation fluid



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Effect of the administration of estradiol-17-β on the incorporation of ^{3}H leucine (A) and ^{14}C serine (B) into the total secreted proteins in the liver cultures. Each point represents the mean of determinations of 3 cultures; the vertical bars indicate the minimum and maximum values observed. 0 represents the experimental starting point (24 h after plating) dotted arrow the moment of estradiol-17-β administration and solid line the moment of hormone removal.

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