Until 48 h, the predominant cell formed is the megaloblast. Although further production of megaloblastic cells gradually ceases after 48 h, existing cells continue to mature and the low enzymatic activity sustained in the blood islands coincided with the continuing presence of megaloblasts. There appeared to be a correlation between the increase in enzymatic activity at 55 h and the appearance of immature normoblasts in the circulation. The dramatic change in the embryo at 55 h may be the result of induction of the de novo pathway for cellular development, and perhaps normoblastic maturation. Embryonic megaloblastosis may, therefore, be related to a metabolic defect in the conversion of orotic acid to uridine monophosphate[5].

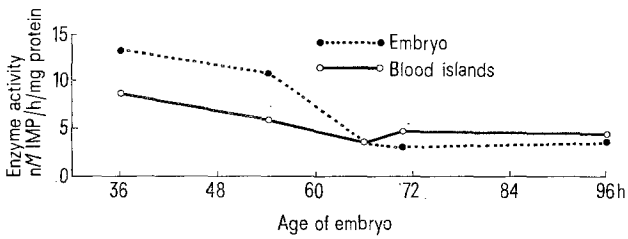

Fig. 2. Hypoxanthine-guanine phosphoribosyl transferase activity in chick embryonic (closed circles) and blood island (open circles) tissues, at different stages of development.

Résumé. La mégaloblastose embryonnaire peut apparaître à la suite d'un défaut métabolique dans la biosynthèse de novo des pyrimidines dans la conversion d'acide orotique à l'uridine 5-phosphate.

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7 April 1972.

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Effect of Spironolactone and Phenobarbital on the Plasma Fibrinogen in Rats

For more than 50 years evidence has been accumulating that fibrinogen is formed in the liver. The microsomes are considered to be the site of the synthesis within the cells while the fibrinogen in the soluble fraction is in storage[1].

Recently, it has been shown that spironolactone (SNL) – formerly known as an antialdosterone steroid without any other hormonal activity – greatly increases the de novo synthesis of several microsomal enzymes, protein, phospholipid, RNA and hemoprotein content in the liver[2,3] and consequently induces resistance in the body against many structurally unrelated exogenous and endogenous toxic substances[4]. Morphologically a marked proliferation of the smooth surfaced endoplasmic reticulum was observed[5].

In order to elucidate further interrelationships we studied the effect of SNL – in comparison with that of phenobarbital – on the synthesis of fibrinogen. That is the reason why the plasma fibrinogen level was measured, since this represents approximately 75% of the total body fibrinogen.

In another series of experiments, C^{14}-labeled amino acids were given and the radioactivity of fibrinogen was determined, in order to exclude the possibility that the increased fibrinogen level was due to a release of fibrinogen stored in compartments which were not in transfer-equilibrium.

Materials and methods. 200 female rats of Wistar strain with a mean body weight of 100 g were divided into 3 groups and treated with SNL or phenobarbital in a dose of 20μM/100 g body wt. twice daily perorally for 3 consecutive days. The first group treated with H_2O served as a control.

For determinations blood was taken on the 4th day after overnight fasting, and liver was quickly removed and weighed. Plasma fibrinogen was determined according to GRANNIS[6] as a clottable protein by adding thrombin in the presence of soybean trypsin inhibitor and ε-amino-

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Effect of spironolactone and phenobarbital on liver weight, plasma fibrinogen level and the incorporation of C^{14} amino acids into fibrinogen

Group	Treatment	Liver weight (g) means ± S.E.	Fibrinogen in plasma (mg/ml)	Radioactivity of fibrin at the time of maximum incorporation (dpm/mg fibrinogen)
1	Control	3.4 ± 0.15	2.6 ± 0.16	6 h after giving C^{14} amino acids 410
2	Spironolactone	4.0 ± 0.08[a]	3.4 ± 0.21[a]	3 h after giving C^{14} amino acids 980
3	Phenobarbital	4.5 ± 0.14[a]	3.9 ± 0.42[a]	33 h after giving C^{14} amino acids 1170

[a] Significantly different from group 1.

n-caproic acid at pH 6.3. After washing carefully the fibrin was resolved in alkaline urea and measured photometrically. As a next step a mixture of 10 uniformly labeled amino acids C^{14} was given to control, SNL or phenobarbital treated rats. In each animal 5 μC/100 g body wt. was given i.p. Blood was taken 2, 3, 6 and 8 h later and the radioactivity of fibrin was measured by using a Packard Tri-Carb liquid scintillation system. The isolation of fibrin has been done as mentioned earlier, but the solubilization of fibrin clit for counting was performed according to ATENCIO and LORAND[7].

Results and discussion. As can be seen in Table I, it is obvious that there is a significant increase of liver weight as well as plasma fibrinogen both in the SNL and phenobarbital treated groups on the 4th day.

The increase is more pronounced in the phenobarbital treated group than in the animals treated with SNL. The incorporation of the labeled amino acids into fibrin isolated from the SNL treated rats is about the double, compared with the control groups and even higher after phenobarbital treatment. The peaks of incorporation in time are also changed both in the SNL and phenobarbital treated groups. In these cases the maximums of radioactivity are at 3 h after giving the labeled amino acids while in controls the peak is at 6 h.

On the basis of our findings we can say that both SNL and phenobarbital are capable of inducing an enhanced synthesis of fibrinogen and this results in an elevated plasma fibrinogen level after treatment for 3 days.

ATENCIO and LORAND[7] have found that a single dose of ACTH given to rabbits caused a similar increase of plasma fibrinogen 24 h later but a far more enhanced incorporation (more than 10-fold increase was noted) even 2 h after giving ACTH. This effect of ACTH seems not be mediated through corticosterone since this steroid did not have any effect either on the plasma fibrinogen or on the rate of incorporation. It is worth mentioning that corticosterone was given in this experiment in a single dose.

Our investigations showed that SNL as a steroidal compound given for 3 consecutive days increased fibrinogen synthesis. This might represent an interesting way of influencing fibrinogen metabolism by steroids. Phenobarbital has the same but more pronounced effect in rats. This fact means that fibrinogen metabolism is able to meet adaptation in large scale and can be influenced by several structurally unrelated substances. Eventually – these drugs known as microsomal enzyme inducers – influenced the synthesis of an enzymatically inactive protein of the liver.

Recently it has been reported by REMMER and CASALS[8] that phenobarbital provokes an enhanced synthesis of the albumin in the liver and this results in an elevated plasma albumin level of rats. The functional and pathological aspects of the elevated fibrinogen level in blood under the effect of inducers are still questioned and unsolved.

Résumé. Par un dosage de 20 μM/100 g poids de spironolactone ou de phénobarbital pendant 3 jours, un niveau de fibrine élevé peut être produit chez des rats. En même temps, l'incorporation des aminoacides C^{14} au fibrinogène est aussi intense. Le phénobarbital est dans tous les cas plus efficace que le spironolactone. Dans notre cas, ces médicaments, connus comme inducteurs des enzymes microsomiques, augmentaient la synthèse protéique, enzymatiquement inactive du foie.

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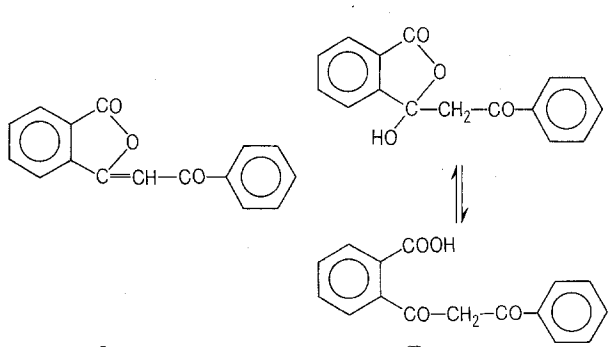
New Inhibitors of Auxin Transport

While investigating the effects of certain lactones on the development of the crucifer *Arabidopsis thaliana* grown under controlled conditions[1], we noted that 3-phenacylidene phthalide I and its hydrate II caused a complete loss in root geotropism when incorporated into the growth nutrient at $2\times10^{-5}M$. The synthetic plant growth regulators 2, 3, 5-triiodobenzoic acid (TIBA) and 1-naphthylphthalamic acid (NPA) are both highly effective inhibitors of auxin transport[2,3] and at low concentrations both abolish the normal positive geotropic response of seedling roots[4,5]. In the present work we have found that I and II are inhibitors of basipetal auxin transport of activity comparable to that of TIBA and NPA.



I II

A quantitative assessment of the effect of I and II on root curvative was made using cress (*Lepidium sativum*) and ryegrass (*Lolium rigidum*) based on the procedure of JONES et al,[5]. Germinated seeds of both species with straight roots 1–2 cm long were transferred to the surface of agar plates containing the compounds under test at various concentrations. The plates were then placed in the dark in a vertical plane such that the initial direction of root growth was horizontal. In untreated plates

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