Table II. Free and conjugated steroids in experiments Nos. 1–6

Experiment No.	Material (time after injection, volume)	Free steroids (Ipm ^3H)	Sulphoconjugated steroids Ipm ^3H (^{35}S)	Steroid-glucuronoside Ipm ^3H (^{14}C)	Total % of injected ^3H-activity
(1)	Bile (1–24 h, 563 ml)	–	28,710 = 5.3 (5,269)	5,510	0.506
	Urine (1–24 h, 1,120 ml)	–	1,779,900 = 2.4 (719,000)	5,300	26.499
(2)	Bile (1–24 h 483 ml)	–	5,084 = 8.4 (603)	1,225	0.262
	Urine (1–24 h, 1,420 ml)	–	727,200 = 2.3 (314,300)	42,150	30.621
(3)	Plasma of ileacal vein (1–120 min, 40 ml)	6,605	127,250	504	0.189
(4)	Bile (1–24 h, 566 ml)	–	24,430	15,110 (0)	0.740
	Urine (1–24 h, 475 ml)	–	56,475	15,070 (0)	1.341
(5)	Bile (1–24 h, 598 ml)	–	42,310	13,990 (0)	6.750
	Urine (1–24 h, 590 ml)	–	12,500	200,100 = 2.07 (97,080)	25.450
(6)	Plasma of duodenal vein (1–30 min, 40 ml)	177,840	577,300	106,390	4.190

noticeable hydrolysis. On the other hand, extensive hydrolysis and reconjugation preceeds the hepatic excretion of steroid glucuronoside, as evidenced by total loss of ^{14}C-activity of the steroid conjugates in bile.

When free 7α-^3H-DHEA was infused into the duodenum (experiment 6), a rapid and complete reabsorption of the steroid could be demonstrated by assay of intestinal venous plasma, 68.2% being conjugated with sulphuric acid and 12.4% with glucuronic acid (Table II). In the remaining fraction of free steroids only the unchanged substrate could be detected. 14% of the ^3H-activity of the steroid conjugates were DHEA-metabolites. While 3β-hydroxy-steroids were exclusively conjugated with sulphuric acid, 3α-hydroxy-steroids were predominantly coupled with glucuronic acid. Since, according to earlier experiments, no qualitative differences were observed in the metabolism of DHEA glucuronoside and DHEA sulphoconjugate (sulphate and sulphatide respectively)[3,5], the injected substrate must have undergone its metabolic changes in the steroid moiety prior to conjugation of the resulting metabolites. The latter processes appear to involve rather specific sulphokinases and glucuronyltransferases, as already demonstrated by Dahm and Breuer[6]. The isolation of small but significant amounts of estrogens as well as of androstendione from the fractions of steroid sulphoconjugates and glucuronosides may also be of interest[7].

Zusammenfassung. Während DHEA-sulfat, welches man bei Versuchspersonen in das Duodenum infundiert hatte, die Darmwand fast ohne Hydrolyse und Metabolismus passierte, erfuhr DHEA-glucuronid eine weitgehende Hydrolyse und Rekonjugation. Freies DHEA wurde hier zu 14% metabolisiert, wobei 3β-Hydroxy-Steroide mit Schwefelsäure, 3α-Hydroxy-Steroide mit Glucuronsäure konjugiert wurden. In der Leber unterliegen DHEA-sulfat und -glucuronid einem direkten Metabolismus.

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Failure of L-Phenylalanine to Prevent Benzene-Induced Pancytopenia

We have previously reported on a group of 60 rabbits that were intoxicated by means of s c. benzene injections[1]. With a dose of 300 mg/kg/day of pure benzene, peripheral pancytopenia could be induced in all the animals within 1–9 weeks. In the above mentioned study it was demonstrated for the first time radioautographically with ^3H-thymidine that the pancytopenia of benzene intoxication is due to a severe inhibition of the DNA-synthesis in the bone marrow cells.

Because of the fact that the chloramphenicol molecule contains a nitrobenzene-ring, one might reasonably as-

sume that this ring, after liberation from the rest of the molecule, is responsible for the well known erythroid depressing action of this drug.

After INGALL's report[2] on the amelioration of the toxic effects of chloramphenicol on the bone marrow by ingestion of phenylalanine, we decided to try it in a group of 10 rabbits, that were subjected to benzene intoxication. These animals were given 300 mg/kg/day of pure benzene s.c. and in addition they were fed 200 mg/kg/day of L-phenylalanine. 2 of the animals died from unexplained reasons shortly after the benzene injections. In the remaining 8 animals peripheral pancytopenia with predominant leukopenia of less than 2000 per mm³ resulted within 3–7 weeks, with an average of $4^1/_2$ weeks. In the group of animals that were given benzene only, the duration until peripheral pancytopenia was induced was 1–9 weeks with an average of 5 weeks.

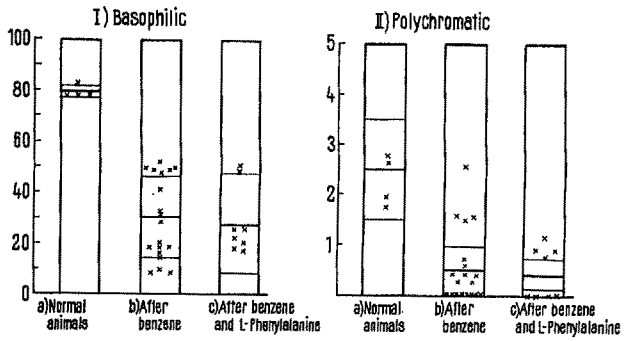
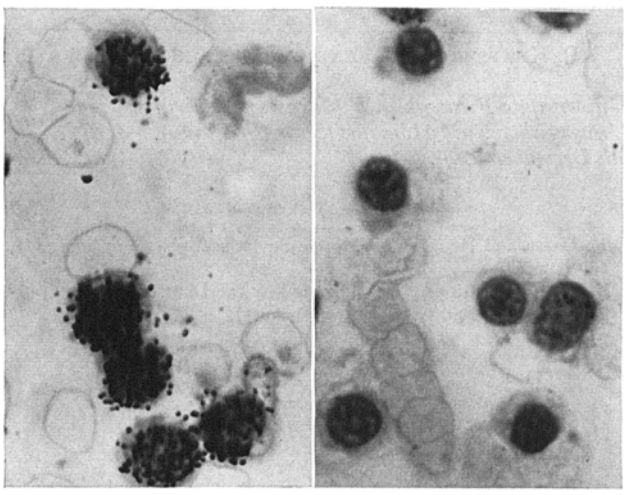


Fig. 1. Note the significant drop of labelling of the basophilic normoblasts (= $K^1/_2$) of 84.5% (S.D. 2.6%) in normal animals to 30.2% (S.D. 16.5%) in the animals on benzene and to 27.7% (S.D. 19.4%) in the animals on benzene and L-phenylalanine. At the stage of the polychromatic normoblast (= $K^1/_4$) the labelling was 2.5% (S.D. 1.05%) in normal animals as compared to 0.52% (S.D. 0.49%) in animals on benzene and 0.43% (S.D. 0.31%) in animals on benzene and L-phenylalanine.



(a) (b)

Fig. 2. (a) illustrates the radioautographic findings of the bone marrow cells of a normal animal. Note the obvious labelling of the erythroid precursors. (b) radioautographic plate from a pancytopenic animal on benzene and L-phenylalanine. Note the almost complete lack of labelling of the red cell precursors.

In vivo radioautographic studies of the bone marrow cells using methyl-^{3}H-thymidine (0.5 μc/g body weight) were performed in 4 normal rabbits, 19 benzene treated animals and 8 rabbits on benzene and L-phenylalanine. The Kodak AR-10 stripping film method was used and the films were exposed for 20 days. In all the animals 500 basophilic and 500 polychromatic normoblasts were enumerated and the % of labelled cells was calculated. Cells were considered to be labelled if the number of grains over their nucleus was more than 3 times the background graincount over an equal area.

In the very young erythroid precursors of the K_2 and K_1 stages according to WEICKER[3] (pronormoblasts and macronormoblasts), no significant difference of labelling was noted between normal and intoxicated animals. The myeloid line was markedly reduced and very difficult to be quantitated. But there was a significant decrease of labelling of the myeloid precursors in the animals receiving benzene and those receiving benzene and L-phenylalanine as compared to normal animals.

Conclusions. After we had been able to induce aplastic anemia in a large series of rabbits by means of s.c. injections of pure benzene in a dose of 300 mg/kg/day, we tried to prevent this condition by feeding 200 mg/kg/day of L-phenylalanine simultaneously. No significant difference was noted in the 2 groups of animals as far as the induction of pancytopenia is concerned. In vivo radioautographic studies with ^{3}H-thymidine disclosed a practically equal decrease of the proliferative potential of all cell lines in the bone marrow in both groups, and in particular a maturation arrest at the stage of the basophilic normoblast (= $K^1/_2$) in the erythroid line. It is concluded that L-phenylalanine is ineffective for the prevention of benzene-induced aplastic anemia.

In the meantime it has been shown that phenylalanine in a dose of 160–230 mg/kg/day failed to reverse the erythropoetic depression induced by chloramphenicol in adults[4,5].

Zusammenfassung. In 2 Gruppen von Kaninchen wurden mittels s.c. Benzolinjektionen aplastische Anämien induziert. Bei der 2. Gruppe wurde gleichzeitig L-Phenylalanin verfüttert. Weder im Auftreten der peripheren Panzytopenie noch in der autoradiographisch erfassten Hemmung der DNS-Synthese waren signifikante Unterschiede vorhanden, und L-Phenylalanin erscheint auch in der Verhinderung der benzolinduzierten aplastischen Anämie wirkungslos.

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