ringen Mengen neben dem körpereigenen Heparin analytisch zu bestimmen, verwandten wir Präparate, die mit S^{35} gekennzeichnet waren und einwandfrei quantitativ nachgewiesen werden konnten.

Zur Herstellung derartiger Präparate wurde S^{35} in Chlorsulfonsäure eingeführt und diese in Anwesenheit von Pyridin mit Xylan umgesetzt. Der so erhaltene Xylanschwefelsäureester wurde Kaninchen intravenös injiziert und der Harn sowie die wichtigsten Organe auf ihre Aktivität geprüft. Infolge der sehr weichen Strahlung von S^{35} war es notwendig, die organische Substanz zu zerstören und das Sulfat als Bariumsalz zu fällen. Die Messungen sind sehr gut reproduzierbar, wie die in Tabelle I zusammengestellten Daten an zwei Versuchstieren mit einem Präparat vom Polymerisationsgrad 12 zeigen.

Tabelle I

Organ	Spezifische Aktivität		Anteil der verabfolgten Aktivität in %	
	I	II	I	II
Leber	8,4	6,8	9,1	9,0
Niere	37,2	46,6	5,3	4,9
Dickdarm	3,5	3,4	1,3	1,8
Dünndarm	3,2	3,2	1,2	1,5
Lymphknoten	15,4	13,8	0,3	0,3

Nach diesen methodischen Vorversuchen prüften wir die Abhängigkeit der Ausscheidung und Speicherung vom Polymerisationsgrad der Präparate. Zu diesem Zweck stellten wir durch Abbau drei Xylanschwefelsäureester gleichen Schwefelgehaltes her, deren Kettenlängen sich wie 10:4:1 verhielten, und untersuchten ihr Verhalten im Organismus in der angegebenen Art. Die Ergebnisse sind in Tabelle II zusammengestellt.

Aus den Daten ergibt sich eindeutig, dass sowohl die Speicherung in den Organen – in besonderem Ausmass in Leber und Milz – als auch die Ausscheidung im Harn erheblich von der Kettenlänge der Xylanschwefelsäureester abhängen. Heparin, das nach seiner Viskositätszahl zwischen Präparat 1 und 2 einzuordnen ist, wird nach MARBET und WINTERSTEIN[1] zu etwa 40% ausgeschieden; es nimmt also keine Sonderstellung ein, sondern verhält sich in dieser Beziehung ähnlich wie die Xylanschwefelsäureester.

Der Deutschen Forschungsgemeinschaft sowie der Dr.-A.-Wander-AG., Bern, möchten wir auch an dieser Stelle für die Unterstützung dieser Arbeit unseren Dank aussprechen.

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Summary

The excretion and storage of xylane sulphuric acid esters which inhibit blood clotting, was investigated in rabbits by using radioactively labelled preparations (S^{35}). A specific storage was shown in different organs (liver, kidney, spleen), the extent of which increased considerably with the grade of polymerisation of the preparations while the excretion was correspondingly less.

[1] Helv. physiol. acta *9*, 24 (1951).

The Occurrence of an Abnormal Black Pigment in the Incisors of Albino Rats Reared on certain Purified Diets[1]

It is known that in albino rats certain vitamin deficiencies, i.e., lack of vitamin A or E, induce incisor depigmentation (whitening), that is, a loss of the normal yellow-brown pigment of the enamel[2]. In these deficiencies also definite histopathological and chemical changes of the incisors have been found[3]. We are herewith reporting the occurrence of an abnormal black pigment in the incisors, which has been previously ob-

[1] Aided by a grant from the "Roche"-Studien-Stiftung.

[2] M. C. SMITH and E. M. LANTZ, J. Home Econ. *25*, 411 (1933). – T. MOORE, Biochem. J. *37*, 112 (1943). – H. GRANADOS and H. DAM, Science *101*, 250 (1945); Proc. Soc. Exp. Biol. Med. *59*, 295 (1945). – H. DAM and H. GRANADOS, Science *102*, 327 (1945).

[3] S. B. WOLBACH and P. R. HOWE, Am. J. Pathol. *9*, 275 (1933). – H. MELLANBY, Brit. Dent. J. *67*, 187 (1939). – I. SCHOUR, M. M. HOFFMAN, and M. C. SMITH, Am. J. Pathol. *17*, 529 (1941). – J. T. IRVING, Nature *150*, 122 (1942). – H. GRANADOS, K. E. MASON, and H. DAM, J. Dent. Res. *24*, 197 (1945); *25*, 179 (1946). – H. DAM, H. GRANADOS, and L. MALTESEN, Acta physiol. Scand. *21*, 124 (1950).

Tabelle II

Nr.	$Z\eta$	Polymerisationsgrad*	Organ	Spezifische Aktivität	Anteil der verabfolgten Aktivität in %
1	0,028	45	Leber	85	21
			Niere	98	6
			Milz	140	0,5
			Lunge	12	0,5
			Darm	10	2,4
			Harn	–	26,0
2	0,0075	12	Leber	7	9
			Niere	40	5
			Milz	10	0,14
			Lymphknoten	15	0,3
			Harn	–	27
3	0,0023	4	Leber	1	0,6
			Niere	10	1
			Harn	–	66

* Die Polymerisationsgrade wurden aus den Viskositätszahlen mit einem K_m-Wert von $6{,}2 \cdot 10^{-4}$ berechnet.

Incidence of pigment changes in the upper incisors of the rats from the two experiments, from the third to the ninth experimental weeks

Groups	First experiment					Second experiment				
	1	1A	2	2A	3	4	4A	5	5A	6
Total number of animals	26	26	26	24	20	28	26	28	27	20
Percentage with depigmentation and black pigment	35	15	58	33	0	0	0	0	0	0
Percentage with black pigment without depigmentation	57	81	42	63	0	54	58	82	52	0
Percentage with normal pigment	8	4	0	4	100	46	42	18	48	100

served by PAUL and PAUL[1] and attributed by them to vitamin A deficiency.

In connection with some experiments that we are carrying out to study certain effects of pantothenic acid, the occurrence of an abnormal black pigment in the enamel of the incisors has been noticed. It has been seen in rats of both sexes, intact as well as adrenalectomized animals, reared on the purified diets referred to below.

This report comprises two experiments (5 groups in each) carried out with young rats weighing between 70 and 80 g. In both experiments the first four groups were given a basal diet containing salt mixture[2] 5 g, casein[3] 20 g, sucrose 64 g, and coconut fat[4] 9 g. Each 100 g of diet were supplemented with 1·9 cm cod liver oil which supplied vitamins A and D. These eight groups received 1% solution of NaCl in distilled water as drinking fluid.

First experiment: Each 100 g of the basal diet given to groups 1 (intact rats) and 1*A* (adrenalectomized rats) were supplemented with 0·3 mg thiamine hydrochloride, 0·3 mg pyridoxin, and 0·9 mg riboflavin. Groups 2 (intact) and 2*A* (adrenalectomized) were fed the same diet as groups 1 and 1*A* supplemented with 43·6 mg calcium pantothenate per 100 g. Group 3 (normal controls) was given a stock colony diet[5] of the following composition: polished rice 48·55 g, corn meal 17·10 g, wheat meal 11·40 g, rye meal 5·70 g, dry meat powder 10·10 g, salt mixture[6] 1·80 g, coconut fat 3·30 g, cod liver oil 1·50 g, dry brewer's yeast 0·60 g, and Vi-De-Sec[7] 0·05 g. Besides, the animals received as a supplement, this one day whole yellow corn and the next day fresh carrots. Furthermore, they received tap water to drink.

Second experiment: Each 100 g of the basal diet given to groups 4 (intact) and 4*A* (adrenalectomized) were supplemented with 1 mg thiamine hydrochloride, 1 mg pyridoxin, 2 mg riboflavin, 10 mg nicotinamide, 30 mg *p*-aminobenzoic acid, 5 mg inositol, 0·05 mg biotin, 0·20 mg folic acid, 100 mg choline chloride, and 5 mg *d,l*-α-tocopherol acetate[1]. Groups 5 (intact) and 5*A* (adrenalectomized) received the same diet as groups 4 and 4*A* supplemented with 43·6 mg calcium pantothenate per 100 g[2]. Group 6 (normal controls) was given the same stock ration as group 3. Groups 1*A*, 2*A*, 4*A*, and 5*A* were adrenalectomized in the fourth experimental week.

In all the eight groups fed with purified rations, the black pigment began to appear from the 2nd to the 3rd experimental weeks, and was found almost exclusively in the upper incisors (with two exceptions, one rat in group 1 and one in group 2*A*, in which animals also a slight black pigmentation of the lower incisors was found). In the groups given the vitamin E-free rations (groups 1 to 2*A*) 15% to 58% of the animals exhibited incisor depigmentation combined with the appearance of the black pigment. The depigmentation was to be expected since these groups received vitamin E-free diets which contained dietary fat, and is in agreement with previous observations[3]. The Table presents the incidence of pigment changes in the upper incisors. In the first experiment 42% to 81% of the rats had black pigment without depigmentation. Contrary to this, none of the animals fed with stock diet (group 3) exhibited any abnormal color change in their incisors. In the second experiment most animals exhibited no incisor depigmentation; however, 52% to 82% of the rats (intact and adrenalectomized) fed with purified rations (groups 4 to 5*A*) had black pigment, whereas none of the animals that received the stock diet (group 6) exhibited any abnormal change of color in their incisors.

The Table shows further that the total incidence of black pigment (taken together when it occurred alone and when combined with depigmentation) in the rats from the first experiment (groups 1 to 2*A*) was appreciably higher than in the second experiment (groups 4 to 5*A*). Furthermore, in the course of the studies it was observed that in the animals of the first experiment the black pigment was deposited to a larger extent than in the rats of the second experiment.

The Figure shows examples of the heads of rats from various groups. The following explanation refers to the color changes in the upper incisors. *A:* normal yellow-brown pigment of the incisors in a rat from group 3. *B:* black pigment in the gingival 1/4 and full depigmentation in the rest of the enamel in a rat from group 1.

[1] H. E. PAUL and M. F. PAUL, J. Nutrition *31*, 67 (1946).

[2] The percentage composition of the salt mixture was 2·31 g ferric citrate, 44·17 g calcium diphosphate, 41·85 g potassium citrate, 11·62 g magnesium citrate, 0·046 g copper sulphate, and 0·0058 g potassium iodide.

[3] The casein used was "Vitaminfreies Casein" from Dr. A. Wander AG., Bern.

[4] "Weisses Cocosnussfett SAIS" from Astra Fett- und Ölwerke AG., Steffisburg.

[5] The diet is prepared daily as follows: First the rice is cooked for a short time in a mixture of water and fresh milk (in the proportion of 4 l of water and 750 ml of milk to 1 kg of rice), and after cooling the other ingredients are added and mixed. The final food has a consistency of gruel.

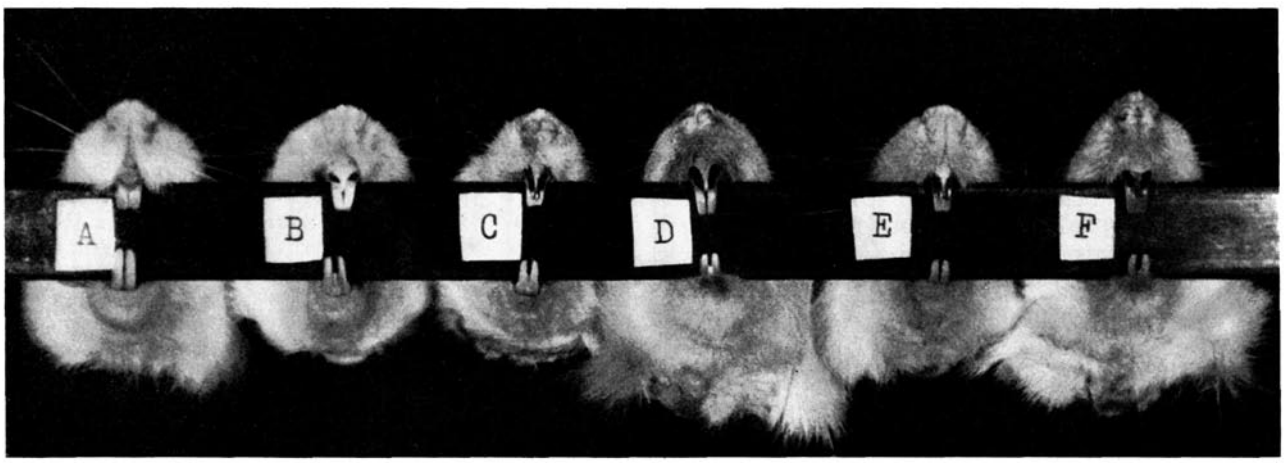
[6] Percentage composition of the salt mixture incorporated in the stock diet: 32·31 g iodinated sodium chloride, 5·14 g magnesium sulphate, 6·50 g monobasic sodium phosphate, 18·30 g dibasic potassium phosphate, 10·40 g tribasic calcium phosphate, 25·00 g calcium lactate, 2·22 g ferric citrate, and 0·13 g manganese sulphate.

[7] Vi-De-Sec is a commercial source of vitamin D from Dr. A. Wander AG., Bern.

[1] The tocopherol acetate was mixed in the cod liver oil.

[2] All the pure vitamins were kindly supplied by F. Hoffmann-La Roche & Co. AG., Basel, for which we express our thanks.

[3] H. GRANADOS and H. DAM, Science *101*, 250 (1945); Proc. Soc. Exp. Biol. Med. *59*, 295 (1945). – H. DAM and H. GRANADOS, Science *102*, 327 (1945).



C: black pigment in the gingival 2/3 and partial depigmentation in the incisal 1/3 in a rat from group 2. *D:* black pigment in the gingival 1/2 and normal yellow-brown pigment in the rest of the enamel in a rat from group 2*A*. *E:* black pigment in the gingival 2/3 and normal yellow-brown pigment in the incisal 1/3 in a rat from group 4. *F:* black pigment in the gingival 1/2 and normal yellow-brown pigment in the rest of the enamel in a rat from group 5.

In both experiments each of the animals fed the purified diets received daily, through the cod liver oil, about 152 units of vitamin A, which should cover amply the requirement of the rat for this vitamin. Therefore it appears doubtful that, as stated by PAUL and PAUL, the black pigment is due to vitamin A deficiency. Experiments are being carried out in order to study this problem.

The following preliminary observations on the characteristics of the black pigment have been made: it disappears rapidly by incineration in the gas flame, slowly in contact with hydrogen peroxide, and very slowly when the teeth are kept in 5% neutral formalin. On the other hand, the black pigment remains the same when the teeth are kept for a long time in 70% ethanol, ether, chloroform, acetone or xylol. Keeping the teeth in boiling water for one hour neither affects the black pigmentation.

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Physiological Institute, University of Basel, January 25, 1952.

Zusammenfassung

Junge Albinoratten beiderlei Geschlechtes, normale und adrenalektomierte Tiere, wurden auf synthetischen Diäten gehalten, in welchen entweder eine Anzahl von Vitaminen fehlte oder welche die Vitamine A, D, E sowie alle chemisch identifizierten B-Vitamine in genügenden Mengen enthielten.

Bei diesen Tieren entstand ein abnormes schwarzes Pigment an den Inzisoren, besonders oben. Kontrolltiere aus der Zuchtdiät zeigten dieses Pigment niemals. Die Häufigkeit in den einzelnen Gruppen ist in der Tabelle wiedergegeben. Die Abbildung zeigt Beispiele.

Are Ba-Ions a Pure Muscular Stimulant on the Rat's Ileum but not on the Guinea Pig's? The Species Difference in Spasmolytic Potency of Some Ganglionic Blocking Agents

FELDBERG[1] recently reported that barium-induced contractions of the guinea pig's and rabbit's ileum were mainly of ganglionic origin, since the induced muscular contractions were partly inhibited by hexamethonium. The concentrations of C_6 which reduced the responses to barium ions had little or no influence on the contractions caused by histamine or acetylcholine. He furthermore suggested that the use of barium salts as a smooth muscle stimulant when testing spasmolytic compounds needed a reinvestigation. FELDBERG's findings that C_6 reduces Ba-induced contractions of guinea pig's and rabbit's ileum, as well as the negligible effect of C_6 on the action of acetylcholine and histamine, have been verified by us. The results are the same if the bath fluid has the composition given by FELDBERG or the composition given below with a $MgCl_2$ content between 5 mg/l and 100 mg/l.

In our experiments we have used ileal preparations from animals killed just before the experiment. The bath fluid was kept at a temperature of 35°C and contained per litre: NaCl 9·2 g, KCl 0·42 g, $CaCl_2$ 0·24 g, NaH_2PO_4 0·1 g, $NaHCO_3$ 0·1 g, $MgCl_2$ 0·005–0·1 g, dextrose 1 g. The pH of this fluid at 35°C is 7·4 when oxygen has been bubbling through the bath for some minutes.

In experiments on rat ileum, however, it was found that hexamethonium had no influence on the contractions caused by $BaCl_2$ (30–80 μg/ml in the bath) or acetylcholine HCl (0·005–0·05 μg/ml in the bath). The concentration of hexamethonium bromide used was 40 μg/ml bath fluid. C_6 was allowed to remain in the bath 2–20 min before the gut was stimulated with Ba or acetylcholine. When hexamethonium in the same concentration was kept in the bath for 2 min, it always completely abolished the contraction otherwise caused by 10 μg nicotine-bitartrate/ml bath fluid.

On the rat ileum this concentration of nicotine caused a contraction of about the same magnitude as the Ba concentrations mentioned above.

Variations of the $MgCl_2$ concentration of the bath fluid from 5–100 mg/l had no influence on the effects of C_6 on rat ileum.

Thus it may be expected that spasmolytics which have slight or no inhibitory effect on the transmission in auto-

[1] W. FELDBERG, J. Physiol. *113*, 483 (1951).