Departure from parallelism	Di-Te-Per adsorbed	Pertussis adsorbed	Pertussis fluid
$S_1-S_2-T_1+T_2$	$0{\cdot}05>P>0{\cdot}02$	$0{\cdot}02>P>0{\cdot}01$	$0{\cdot}4>P>0{\cdot}3$

cant difference between the pertussis or Di-Te-Per vaccines with addition of aluminium phosphate and the fluid vaccines. Thus our hypothesis that we were assaying two samples of the same active substance was contradicted by the results.

Should further experiments show that an addition of aluminium phosphate to pertussis vaccines might influence the degree of immunity against whooping-cough, and that in such a case aluminium phosphate does not behave as an inert substance but influences the dose-response regression lines, difficulties might arise in expressing the value of a vaccine containing aluminium phosphate in terms of an international standard that would not contain aluminium phosphate. The difference in protection between the same series of a pertussis vaccine with and without addition of aluminium phosphate would depend on the dose used for comparing the vaccine. In our case, the difference was very slight with a larger dose, whereas with a smaller dose it proved to be three times in favour of the vaccine with an addition of aluminium phosphate. An addition of diphtheria and tetanus toxoids to a pertussis vaccine did not influence the dose-response regression lines.

Acknowledgment. – The author is indebted to J. BENKOVIĆ, LJ. MANDIĆ, N. KÖHLER, and N. ŠKARICA for their help in this work.

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Institute for the Control and Research of Immunobiological Substances, Zagreb (Yugoslavia), July 9, 1957.

Zusammenfassung

In Laboratoriumsuntersuchungen wurde der Einfluss von Aluminiumphosphat auf die «dose-response regression lines» des Impfstoffes gegen Keuchhusten untersucht.

Multiple Dietary Necrotic Degeneration in the Mouse

A fatal deficiency disease, multiple necrotic degeneration, can be produced in mice by feeding a semi-synthetic diet which is based on dried American *Torula* yeast as the sole source of protein[1]. The ration which has been used for the production of liver necrosis in rats[2], is low in cystine, and simultaneously deficient in two essential dietary factors, namely, vitamin E, and Factor 3[3]. The pathology of multiple necrotic degeneration in the mouse is characterized by extensive necrotic changes in the heart muscle, liver, kidneys, and peripheral muscle (Fig. 1). The massive necrosis of the heart predominates. The changes are associated with a pronounced pancreatic atrophy and with degeneration of the testes. In the present studies, weanling, male F1 progeny from the cross between female BALB/cAnN and male DBA/2JN strains of homozygous mice was used[4]. The mice, weighing between 8 and 15 g at weaning, were kept in individual wire-mesh bottom cages at a constant temperature of 23° to 25°C. Diets were fed *ad libitum*. Supplements (Factor 3, vitamin E, or cystine) were mixed into the dry basal ration. Control mice were maintained on a semi-purified diet containing 30% casein.

The outcome of five successive experiments (*A–E*) is presented in the Table. Death occurred on the basal diet between the 38th and 96th day. The average survival times of these groups ranged from 65 to 73 days. The overall incidence of necrotic lesions in different organs was: Heart 91, liver 54, muscle 47, and kidneys 42%. Multiple necrotic degeneration appears to develop in stages: Between the 38th and the 50th day necrotic lesions were found only in the heart. The organ was frequently enlarged. The valves and the endocardium were normal. After 50 days liver necrosis was seen as well. This lesion was indistinguishable in appearance from dietary liver necrosis in the rat, but it did not precipitate the acute, terminal breakdown seen in the latter species. The majority of mice surviving for 68 or more days also had skeletal muscle degeneration (muscular dystrophy), kidney lesions, and atrophy of the pancreas. The kidneys were enlarged and showed white spots uniformly distributed over their surface. Occasionally hematuria was noted. The pancreatic atrophy was restricted to the secretory portion of the gland (Fig. 2). The picture was reminiscent of the acinar atrophy observed in kwashiorkor[5]. In the latter stages of multiple necrotic degeneration there was also atrophy of the testes.

Either Factor 3 or vitamin E alone prevented multiple necrotic degeneration in the mouse (Table). Cystine had only a partial effect[6]. The protective effects are in good correlation with those obtained against liver necrosis in the rat. The Factor 3 concentrate used in experiment *D* was prepared by fractionation from brewers yeast. It prevented the gross pathological changes for an extended time[7]. The observed protective effects were paralleled by significant differences between the growth rates of the various groups.

Factor 3, described as an independent dietary agent in 1951 by SCHWARZ[8], is water soluble, strongly bound to protein and organic in nature. It has been obtained from various natural sources in high concentration[9]. Recently, Factor 3 has been shown to contain *selenium* in organically bound form[10]. It is extremely potent for preventing liver necrosis in the rat, and exudative diathesis in the chick[11]. Factor 3 can be replaced by

[1] General composition: Sucrose 59, *Torula* yeast 30, vitamin E-free lard 5, salts 5, and a vitamin mixture (in lactose) 1. Vitamin A acetate (1 mg%) and vitamin D_2 (1 μg%) are added.

[2] K. SCHWARZ, Proc. Soc. exp. Biol. Med. *77*, 818 (1951).

[3] K. SCHWARZ, Ann. N. Y. Acad. Sci. *57*, 878 (1954). – The term 'Factor III' has more recently been used for a form of vitamin B_{12} [W. FRIEDRICH and K. BERNHAUER, Angew. Chem. *65*, 627 (1953)]. There is no relation between Factor 3 against necrotic liver degeneration and the vitamin B_{12}-Factor III.

[4] The cooperation of the Animal Production Section, National Institutes of Health, is gratefully acknowledged.

[5] H. C. TROWELL, J. N. P. DAVIES, and R. F. A. DEAN, *Kwashiorkor* (Edward Arnold Ltd., London 1954).

[6] The effect of L-cystine has been found to be caused by a trace contamination with selenium. K. SCHWARZ, unpublished results.

[7] With the exception of the testicular atrophy.

[8] K. SCHWARZ, Proc. Soc. exp. Biol. Med. *78*, 852 (1951).

[9] K. SCHWARZ *et al.*, unpublished results.

[10] K. SCHWARZ and C. M. FOLTZ, J. Amer. chem. Soc. *79*, 3292 (1957).

[11] K. SCHWARZ, J. G. BIERI, G. M. BRIGGS, and M. L. SCOTT, Proc. Soc. exp. Biol. Med. *95*, 621 (1957).

Multiple Necrotic Degeneration in the Mouse (Summary of five experiments)

Diet	Experiment	Average weight gain in 8 weeks (in g)	Duration (in days)	Number of		Incidence of necrotic degeneration in			
				Animals	Deaths	Heart	Liver	Muscle	Kidney
Control (casein diet) . .	*A*	15·2	200	21	0	0	0	0	0
	B	14·5	140	15	0	0	0	0	0
	C	14·2	79	5	0	0	0	0	0
	Totals	14·7	—	41	0	0	0	0	0
Basal (*Torula* yeast diet)	*A*[1]	6·2	77	15	10[2]	—[3]	10	6	10
	B	8·2	96	16	11[2]	15	11	6	6
	C	6·1	86	10	10	9	3	5	3
	D	6·8	81	15	12[2]	12	7	6	8
	D[1]	8·5	82	23	14[2]	22	12	14	6
	Totals	7·4	—	79	57[2]	58[3]	43	37	33
	In %:				*100*	*91*	*54*	*47*	*42*
Basal + Factor 3[4] concentrate	*D*	11·1	129	19	0	0	0	0	0
Basal + vitamin E[5] . . .	*E*	10·5	114; 188[7]	26	0	0	0	0	0
Basal + cystine[6]	*E*	12·1	114; 188[8]	27	0	25	0	0	1
	In %:				*0*	*93*	*0*	*0*	*4*
Basal + vitamin E[5] and cystine[6]	*B*	14·3	273	19	0	0	0	0	0

[1] Vitamin A and D supplement omitted in these groups.

[2] There were no survivors. A total of 22 out of 79 animals were sacrificed during the experiment, between the 54th and the 72nd day, for histological and other studies.

[3] Incidence of heart lesion not registered in experiment *A*.

[4] Factor 3 concentrate prepared from an enzymatic digest of brewer's yeast, equivalent to 40 units of Factor 3 in 100 g of diet[3].

[5] 50 mg% DL-α-tocopherol acetate.

[6] 0·5% L-cystine.

[7] 13 mice killed at 114 days and 13 killed at 188 days after weaning.

[8] 13 mice killed at 114 days and 14 killed at 188 days after weaning.

certain other selenium compounds, including inorganic selenite[10]. In the mouse multiple necrotic degeneration was prevented by 15 μg of the element (as Na_2SeO_3) in 100 g of the basal diet: All 18 animals of the selenium supplemented group survived for 100 days and were normal at autopsy; a simultaneous control group of 33 mice on the basal ration had an average survival time of 69 days, with no survivors.

The main pathological elements, i.e., heart lesions, liver necrosis, kidney degeneration and muscular dystrophy, which occur *jointly* in multiple necrotic degeneration in the mouse have been observed *separately*, in different species, on vitamin E deficient diets[11]. Heretofore, most of these lesions were attributed solely to lack of tocopherol. Our results show clearly that necrotic degeneration is of multiple dietary origin. It can occur only when both Factor 3 (i.e., biologically active selenium) and tocopherol are missing simultaneously. It is conceivable that the various necrotic lesions result from the disturbance of an essential metabolic mechanism which is dependent on Factor 3 and tocopherol, and common to all organs involved.

[11] K. E. Mason, in W. H. Sebrell and R. S. Harris, *The Vitamins*, Vol. III, 545 (Academic Press, New York, 1954).

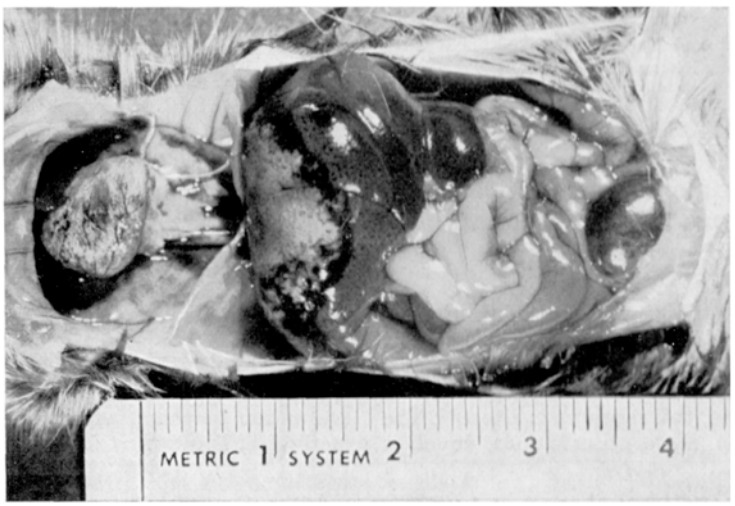

Fig. 1. Multiple dietary necrotic degeneration. Note necrotic lesions of heart and liver, and also discoloration of urine in bladder (hematuria).

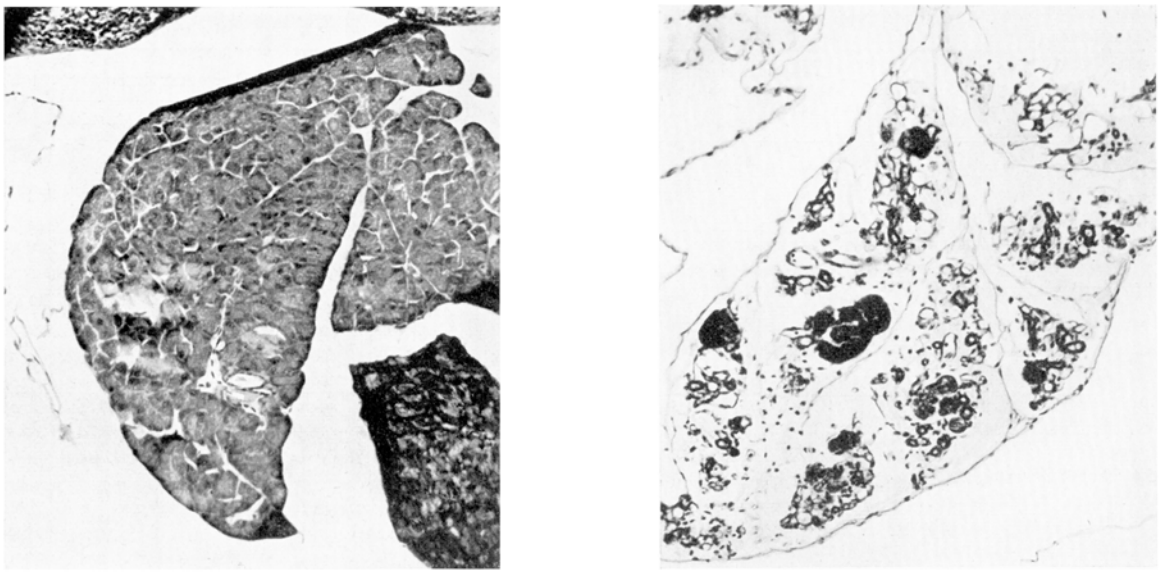

Fig. 2. Pancreatic atrophy. Left: control; right: after 80 days on the basal diet. Paraffin, H. E.

The authors are indebted to Dr. GEORGE L. FITE for histological examinations, and to Mr. RODNEY H. DUVALL for technical assistance.

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National Institutes of Health, U. S. Public Health Service, Bethesda (Maryland), May 21, 1957.

Zusammenfassung

Bei der Maus wird durch eine Diät eine tödliche Mangelkrankheit, sogenannte multiple, nekrotische Degeneration, erzeugt. Diese Diät ist arm an Cystin und frei von Faktor 3 und Vitamin E. Nekrosen des Herzmuskels stehen im Vordergrund, ausserdem kommt es zu Degeneration von Leber, Nieren und Muskulatur. Pankreas und Testes zeigen Atrophie bei gestörtem Wachstum. Die Krankheit wird entweder durch den Faktor 3 oder durch Vitamin E verhütet. Der Faktor 3, der vor kurzem als eine organische Selenium-Verbindung identifiziert wurde, ist durch Selenit-Selenium ersetzbar.

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The Effects of Secretin on Urinary Volume and Electrolytes in Normal Subjects and Patients with Chronic Pancreatic Disease

It is well established that intravenous injection of secretin into normal subjects stimulates the release of a large volume of alkaline pancreatic juice containing a high concentration of sodium bicarbonate, whereas in chronic pancreatic disease, this response is altered[1]. The Secretin Test, based on these observations, involves duodenal intubation[2]. In an attempt to avoid the difficulties of intubation, we have investigated changes in *urinary* excretion of sodium and alkali following secretin injection, in normal subjects and patients with chronic pancreatic disease. Patients were included only if the diagnosis was supported by the most critical clinical and biochemical evidence.

The following experiments were performed:

(a) *13 Normal Subjects:* control injection of normal saline, followed within a week by secretin injection.

(b) *19 Normal Subjects:* secretin injection only.

(c) *7 Patients with severe chronic pancreatic disease:* secretin injection.

Most of the normal subjects were medical students.

Procedure: *Day before test:* adequate fluid intake (2–3 l).

Day of test. Subject fasting, *7.30 a.m.* drinks 100 ml water. Continues to drink this volume at half-hourly intervals throughout the test.

8.30 a.m. Subject at rest in a chair.

9.00 a.m. Subject empties bladder. This specimen is discarded. Test begins: Bladder emptied at *9.20 a.m.*, *9.40 a.m.*, *9.50 a.m.* and *10.00 a.m.* and specimens kept separately under toluene.

10.01 a.m. Injection of test material. Bladder emptied at *10.10 a.m.*, *10.20 a.m.* and *10.30 a.m.*

The injection material was *either* Secretin (Lilly) at a dose of 1 clinical unit/kg body weight, in normal saline, at a concentration of 5 units per ml, *or* an equal volume of normal saline. Both injections were intravenous, and given over 2 min.

The principal measurements made on the specimens were:

(1) *Volume.*

(2) *Sodium* – by external standard flame photometry.

(3) *Titratable Acidity and Bicarbonate.* Separate estimation of these factors was avoided by using the method of DAWSON *et al.*[3], which measures, in a single

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