Table II
Enzymatic Hydrolysis of Yeast Nucleic Acids by the H_2SO_4-treated Enzyme at pH 5·6 (*A*); at pH 8·6 (*B*); by Crystalline Ribonuclease (*C*); by *A* + *C*. Acid Phosphatase Added to all Digestion Mixtures for 3 h (pH 5·6).

	% *I.P.* based on the total P of substrate
A	50–55%
B	8–10%
C	50%
A + *C*	72–75%

hydrolytic activity towards either bonds, those involving purine as well as pyrimidine nucleotides. The *I. P.* in excess of 50% in the experiments in which crystalline ribonuclease plus partially inactivated liver ribonuclease were used must come from purine nucleotides. Therefore the other 25–28% of *I. P.* in the experiments in which only the partially inactivated enzyme was used, must come from pyrimidine nucleotides.

As final products of hydrolysis, using yeast nucleic acids as well as cyclic nucleotides as substrates, we found purine and pyrimidine mononucleotides esterified at the 2 and 3 position of the ribose moiety, their relative proportion varying from 40–60% at either pH. (The acid monoesterase was inhibited by 0·03 *M* fluoride, the alkaline by 0·05 *M* arsenate.) We employed COHN's method[8] for their identification. No isomerase activity could be detected in our preparations.

The two kinds of split products suggested the presence of two different enzymatic activities in our preparations.

Following the procedure of DAVIS and ALLEN[9] we absorbed the enzyme preparation on IRC–50 which had been equilibrated with 0·1 *M* acetate buffer at pH 6·0. The concentrated filtrate (1 cm^3 = 1 g of liver tissue) cleaved nucleic acid only to a slight degree (about 5 to 10%); the cyclic nucleotides were found to be split to 2 nucleotides to about 85%, the remaining 15% being 3′ nucleotides. Activity was about the same at pH 5·6 and 8·6.

The column was then washed with 1 *M* acetate buffer at pH 6·0, and the concentrated eluate was found to cleave yeast nucleic acid to 3′ nucleotides to the extent of 90%, the remaining 10% being 2′ nucleotides at pH 5·6. The same hydrolysis products were obtained with cyclic nucleotides as substrates. At alkaline pH no appreciable activity was observed.

It might well be that the alkaline ribonuclease was inactivated by this procedure; on the other hand one should recall the finding of WILLSTÄTTER[10] that impure gastric lipase acted best at pH 4–5, while after purification optimal action was observed at pH 8. Apparently removal or changes of different contaminating proteins may markedly influence the pH activity curve of an enzyme.

F. STECKERL, JOANN KING, and A. OFODILE

Department of Biochemistry, Boston University School of Medicine, Boston (Mass.), June 18, 1957.

[8] W. E. COHN and E. VOLKIN, Nature *167*, 483 (1951).

[9] F. F. DAVIS and F. W. ALLEN, Biochim. biophys. Acta *21*, 14 (1956).

[10] R. WILLSTÄTTER, E. WALDSCHMIDT-LEITZ, and F. MEMMEN, J. physiol. Chem. *125*, 93 (1923).

Zusammenfassung

Die Befunde zeigen, dass am Abbau von Hefenukleinsäure durch Rattenleberextrakte zwei Fermente wirksam sind: *a*) eine Ribonuklease, welche 3-Nukleotide als Spaltprodukte liefert, und *b*) eine Phosphodiesterase, welche zyklische Nukleotide zu 2-Nukleotiden abbaut.

The Dose-Response Regression Lines of Pertussis Vaccines with and without Aluminium Phosphate

We tested a combined diphtheria-tetanus-pertussis vaccine adsorbed on aluminium phosphate, a pertussis monovaccine adsorbed on aluminium phosphate, and a pertussis monovaccine without addition of aluminium phosphate. The same batch of pertussis vaccine was used in both monovaccines and in the combined diphtheria-tetanus-pertussis vaccine. In our experiments, we made use of the active mouse-protection test and examined the dose-response regression lines of the fluid and adsorbed pertussis vaccines.

Since each vaccine series used in our experiments contained the same amount of active substance of the *H. pertussis* germ, it might have been expected that there would be no significant departure from parallelism of the dose-response regression lines, and that the degree of protection afforded by all three series would be almost the same.

As one of our vaccine series contained aluminium phosphate and the other had been prepared without aluminium phosphate, we were in a position to observe the influence of aluminium phosphate on the dose-response regression lines. On the other hand, as one of the vaccine series contained both aluminium phosphate and diphtheria and tetanus anatoxins, and the other only aluminium phosphate, we had the opportunity of observing the influence of diphtheria and tetanus anatoxins on the dose-response regression lines as well.

Results. – Since an international standard vaccine against whooping-cough is to be adopted, and the value of the various vaccine series is to be expressed in terms of the international standard, the influence of aluminium phosphate not only on the degree of immunity against whooping-cough but also on the dose-response regression lines of vaccines, with and without aluminium phosphate, is worthwhile observing.

An addition of aluminium phosphate changes the dose-response regression lines of diphtheria toxoid in such a way that they are not parallel with the dose-response regression lines of a prophylactic without aluminium phosphate (HOLT[1]). The same occurs with an addition of aluminium hydroxide (JERNE and WOOD[2]).

The following table shows a statistical analysis of the departure from parallelism of the dose-response regression lines of all the three vaccines tested, compared with the reference (pertussis fluid) vaccine prepared at our Institute. The tests were repeated six times.

The statistical analysis of the departure from parallelism of the dose-response regression lines between the pertussis fluid vaccine and the reference fluid vaccine showed no significant difference, but there was a signifi-

[1] L. B. HOLT, *First European Meeting of Biological Standardisation – Diphtheria Toxoid* (Lyon 1955).

[2] N. K. JERNE and E. C. WOOD, *Biometrics*, American Statistical Association *5*, 273 (1949).

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.

D. IKIĆ

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).