A similar picture is obtained from the figures dealing with the *synergist* activities of the carbinols. In the case of *susceptible* flies, their effect is small (lowering the value of 0·7 for DDT to 0·3–0·4), whilst for the *resistant* strain the 'active' carbinols increase the toxicity of DDT about 10 times, the 'inactive' ones much less. The distinction between the two groups is clear only in the latter case.

Replacement of the hydroxyl group in the carbinols by hydrogen (compounds II–V) produces compounds which are less active than DDT in susceptible and somewhat more active than the standard compound in resistant flies. If in the latter case the differences are significant, it appears that the sequence of the activities is the reverse from what one would have expected on the basis of the 'trihedral theory': $CF_3 > CF_2Cl > CFCl_2$, whilst the size of the halogenated methyl group varies accordingly to $CFCl_2 > CF_2Cl > CF_3$. This is true both for the insecticidal power and the synergist activity of the compounds.

Acetylation of the hydroxyl group in the 'active' carbinols (XIV–XVI) destroys the activity for the fluorodichloro and—to a lesser degree—for the difluorochloro compound. The trifluoro compound (XIV) has the same effect as the free carbinol. It can be assumed that in the latter case the esterases of the body are capable of hydrolyzing the acetyl compounds. Indeed, XIV reaches its activity only after 72 h. With increasing size of the methyl substituents, the hydrolysis becomes more difficult—perhaps as a result of steric hindrance. One would then expect that the acetate of di-(*p*-chlorophenyl)-trichloromethyl-carbinol would be completely inactive.

The mode of action of the 'active' carbinols is not quite clear. They may function as unspecific narcotics, as proposed by GAVAUDAN and POUSSEL[8] for DDT. In this respect, it may be recalled that the vapour of di-(*p*-chlorophenyl)-trifluoromethyl-carbinol has a certain depressant effect on flies[9]. Experiments in this direction are now in progress.

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Résumé

L'influence de la halogénuration du groupement méthyl dans le di-(*p*-chlorophényl)-méthyl-carbinol sur l'activité de ce composé comme insecticide ou synergiste du DDT a été étudiée. On essaye de donner une explication rationnelle des faits observés.

[8] P. GAVAUDAN and H. POUSSEL, C. r. Acad. Sci. *224*, 683 (1947).
[9] S. COHEN and A. S. TAHORI, J. Agr. Food Chem. *5*, 519 (1957).

The Enzymatic Degradation of Yeast Nucleic Acid by Normal Rat Liver Tissue[1]

Using rat liver homogenates and mitochondrial preparations respectively, DELAMIRANDE *et al.*[2] and ROTH[3] found two optima in measuring the pH activity of their preparations against yeast nucleic acids. On the basis of these findings they postulated the presence of an acid as well as of alkaline ribonuclease in normal rat liver tissue. MAVER and GRECO[4] reported similar results. According to ROTH[5] the alkaline ribonuclease is similar or identical with the crystalline pancreatic ribonuclease, whereas the peak on the acid side is due to the action of a non-specific diesterase.

Table I
Enzymatic Hydrolysis of Yeast Nucleic Acid by Rat Liver Ribonuclease Plus Prostate Acid Phosphatase

pH	% *I.P.* Based on the total P of substrate
5·2–8·9	93–100%

Applying to normal liver homogenates the same method of extraction[6] which yielded a soluble enzyme preparation in the case of the cells of the mouse ascites tumor, we confirmed the presence of two peaks in the pH activity curve using 0·14 veronal buffer (1–2 cm^3 rat liver extract corresponding to 1–2 g rat liver; 10 mg yeast nucleic acid: Total volume 10 cm^3). Maximal depolymerization as measured by determination of the acid soluble P, after precipitation with MacFadyen's reagent, was observed at pH 5·6 and 8·6 respectively after 6–8 h at 37° C. Moreover the rate of hydrolysis at these pH optima was found to be approximately the same. After exhaustive digestion (40–48 h) all the diester bonds of yeast nucleic acid were found to be cleaved, as suggested by the amount of *I. P.* after 3 h incubation with purified prostate acid phosphatase at pH 5·6 (Table I). Therefore, it does not seem that this 'alkaline' ribonuclease can be identical with or even similar to pancreatic ribonuclease, since pancreatic ribonuclease is known to hydrolyze linkages in yeast nucleic acid involving pyrimidine nucleotides only, and not all diester bonds. Moreover, purine polynucleotides (prepared by exhaustive digestion of yeast nucleic acid with crystalline ribonuclease) were also found to be cleaved completely by this preparation, and the pH activity curve showed the same two optima at pH 5·6 and 8·6 respectively.

In connection with the above results the observation of HIRS *et al.*[7] are of interest. Their chromatographic analyses of acid extracts of beef liver did not reveal the presence of either of the two enzymes of the pancreas with ribonuclease activity.

Our preparations apparently contain only ribonuclease and no non-specific diesterase activity since glycerolphosphorylcholine was not cleaved at any pH.

Still, the two peaks in the pH activity curve seem to be due to the presence of two ribonucleases. Treatment of the enzyme preparation with sulfuric acid (standing in 0·25 *M* sulfuric acid for 24 h, at 5°C) revealed a difference in its hydrolytic behaviour at pH 5·6 and 8·6 respectively (Table II). There was no appreciable cleavage at pH 8·6. The partial inactivation at pH 5·6 involved reduction to about the same degree of the

[1] This work was supported by a grant (C-2459C2) from the U. S. Department of Public Health.
[2] G. DELAMIRANDE, C. ALLARD, H. C. DA COSTA, and A. CANTERO, Science *119*, 351 (1954).
[3] J. S. ROTH, J. biol. Chem. *208*, 181 (1954).
[4] M. E. MAVER and A. E. GRECO, J. nat. Cancer Inst. *17*, 503 (1956).
[5] J. S. ROTH, Fed. Proc. *15*, 341 (1956).
[6] F. STECKERL, Arch. Biochem. Biophys. *58*, 73 (1956).
[7] C. H. W. HIRS, W. H. STEIN, and ST. MOORE, Congr. int. Biochem. Résumés communications 2e Congr. (Paris 1952), p. 258.

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.

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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).