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ANTIVIRAL ACTION OF DERIVATIVES OF ω-AMINOACETOPHENONE

BY

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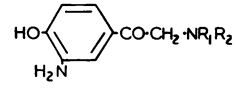
Numerous structural analogues of 3-amino-4-hydroxy-ω-methylaminoacetophenone were tested for their effect on the multiplication of influenza virus (FM_1 strain) in embryonated eggs, infected via the allantoic cavity. Antiviral activity was found in ω-aminoacetophenones containing an amino and hydroxyl group in the aromatic nucleus in the *ortho* or *para* positions to each other. The most powerful antiviral activity was found in the series of ω-alkylamino-5-amino-2 : 4-dihydroxyacetophenones. Derivatives of acetophenone with other substituents in the aromatic nucleus or in the aliphatic chain were without activity. *In vitro*, several analogues inactive in the egg test, as well as those which were active, exerted a virucidal and to a lesser extent a haemagglutinin-destroying action. Antiviral action in the allantoic test could not be prevented or inhibited by simultaneous administration of reducing substances, or of some amino-acids or vitamins. No inhibition of virus multiplication occurred in embryonated eggs infected via the yolk sac, or in mice infected intranasally with FM_1 virus. The activity in the allantoic test could be explained by the virucidal action of the compounds on the virus present in the allantoic fluid. No satisfactory interpretation of the empirical relationship between chemical structure and antiviral activity could be found.

In recent years numerous substances with antiviral properties have been reported. There are excellent reviews of this field by Matthews and Smith (1955) and Hurst and Hull (1956). However, none of the substances hitherto found to inhibit the multiplication of viruses in experimental host systems deserved clinical trial. The larger part of the investigations reported aimed at finding compounds interfering with the multiplication of the influenza viruses. These viruses were preferred for therapeutic trials because they are easy to handle and their multiplication can be determined fairly well. Moreover, the diseases caused by the influenza viruses have important medical and economic aspects.

In spite of much progress in the study of the multiplication of the influenza virus, the insight gained is not yet adequate for the establishment of a relationship between chemical structure and activity in antiviral substances. Therefore, it is still worth while to test compounds chosen arbitrarily for anti-influenzal activity. Screening tests of several hundreds of such compounds revealed that 3-amino-ω-*tert.*-butylamino-4-hydroxyacetophenone (A-5) inhibited the multiplication of influenza virus in embryonated hen eggs. A number of derivatives of phenol, benzoic acid and acetophenone having common structural features with A-5 proved to be inactive. This paper reports the results of an investigation intended to find other, possibly more active, derivatives of acetophenone. The empirical relationship between chemical structure and antiviral activity and the mode of action of these inhibitors of viral multiplication were also studied. The results obtained with A-5 suggested investigation of compounds with the general formula:



Group A

In this formula R_1 and R_2 represent a hydrogen atom or an alkyl, *cyclo*alkyl, or arylalkyl group. The other compounds of which the antiviral activity was investigated are structural analogues of these members of group A. Attention was paid to the influence of other substituents in the aromatic nucleus and to variation of the aliphatic chain.

METHODS AND MATERIALS

Virus.—The FM_1 strain of influenza A' virus used (World Health Organization Notation Influenza A-USA-47) had between 30 and 90 mouse passages and between 1 and 40 egg passages. We prepared our seed virus by the usual methods (Liu, Malsberger, Carter, Sanctis, Wiener, and Hampil, 1957).

Eggs.—Embryonated eggs of White Leghorns or Rhode Island Reds incubated for 11 days at 37° were used.

Virus Determination

Haemagglutination Titre.—The haemagglutination titre (HA) of an allantoic fluid was determined as described by van de Veen (1950). The titre is expressed as the logarithm of the final dilution with complete or partial haemagglutination. When calculating the logarithm, the interval between two successive two-fold dilutions was divided into four equal logarithmic intervals. Values lower than 1.2 were not determined exactly.

Infectivity.—Infectivity titrations were carried out as described by Rasmussen and Stokes (1951). The negative log ID50 was calculated by the method of Reed and Muench (1938). The standard deviations of the values so obtained were calculated according to Pizzi (1950).

Compounds

Details of the synthesis of the hydrochlorides and sulphates of the ω-aminoacetophenones are given elsewhere (Brug, 1958). The compounds were injected into the allantoic cavity in 0.2 ml. of a solution of 2, 1, 0.5, or 0.25×10^{-4} mole/ml. (4, 2, 1, and 0.5×10^{-5} mole/egg). Whenever possible they were dissolved in the required concentration in saline. If necessary HCl or NaOH was added. A *p*H near 7 was preferred but not obligatory. Solutions were sterilized by filtration. Poorly soluble compounds were suspended in 2% sterile carboxymethylcellulose gel. These manipulations were performed in sterile mortars under ultra-violet light in an aseptic cabinet.

Routine Test

For each concentration of the compound 6 eggs were injected with the solution or suspension mentioned above, in the allantoic sac. After 1 hr. the eggs were inoculated with 0.1 ml. of a 10^{-4} dilution of a virus suspension with log haemagglutinin titre $\geqslant$ 2.4. After incubation for 48 hr. at 36° the eggs were candled and dead ones discarded. 1 ml. portions of the allantoic fluid of each egg were harvested and pooled for each group. The haemagglutinin titres of these pools were determined. If doses of 0.5×10^{-5} mole/egg were too toxic, we tested lower concentrations. A compound was considered active and investigated further if in repeated experiments the difference between the log haemagglutinin titre of allantoic fluid from control eggs and the log titre of allantoic fluid from eggs treated with the compound under trial was 0.6 or more.

In Vitro *Tests*

Compounds giving a positive result in the routine test were investigated *in vitro*. The compound was dissolved in virus-containing allantoic fluid of high titre in a concentration equal to the lowest inhibitory concentration in the egg; the volume was taken as 10 ml., being the approximate amount of allantoic fluid in the egg. The log haemagglutinin titre and negative log ID50 of this mixture were determined immediately after mixing, and after 5, 24, 48, and occasionally 72 hr. incubation at 37°. As a control we used the same virus-containing allantoic fluid diluted with an equal volume of saline.

RESULTS

pH of Injected Solutions and Virus Multiplication

Most compounds were brought into solution by adding an equivalent of HCl or NaOH. Some of the resulting solutions, as well as those of some acid or basic substances, had *p*H values below 5 or above 8. The buffering capacity of allantoic fluid in the region of *p*H 6 to 7 was determined to be about 0.008 van Slyke units. This value is in accord with the estimate obtained by Candles and Romanoff (1954). The low value of the buffering capacity made it necessary to check the influence of *p*H on the multiplication of influenza virus. To lower the *p*H of allantoic fluid a value near 6, about 1.5×10^{-5} equivalents of HCl must be added to 1 ml. of allantoic fluid. In the normal egg tests, we added no more than 0.4×10^{-5} equivalent of acid ml. of allantoic fluid. Fauconnier (1955) found that the multiplication of influenza A (PR 8 strain) in embryonated eggs was inhibited if the *p*H of allantoic fluid was 6 or less. Alkaline media also restrict growth of this virus (Ruffilli, 1955). Injection of 0.2 ml. saline made acid (*p*H 2 to 4) or alkaline (*p*H 10 to 12) by HCl or NaOH had no significant influence on the multiplication of influenza FM_1 as measured by the haemagglutinin titre of allantoic fluid. The mean difference between the log haemagglutinin titres of experimental and control fluids was about 0.15 units in 32 experiments over the ranges stated.

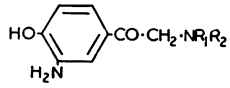
Antiviral Activity

The effects of many derivatives of acetophenone substituted in the aromatic nucleus and in the aliphatic chain on the concentration of influenza FM_1 haemagglutinin in the allantoic fluid of infected eggs were determined. The compounds which caused a significant decreased concentration of haemagglutinin after an incubation period of 48 hr. are listed in Table I.

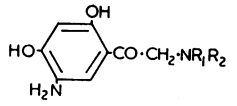
TABLE I

ANTIVIRAL ACTIVITY OF ω-AMINOACETOPHENONES

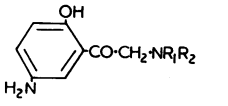
0·2 ml. of a solution of the compound was injected in allantoic cavity of 6 embryonated eggs. After 1 hr. eggs were infected with 0·1 ml. seed virus (influenza FM_1). After incubation for 48 hr. at 36° haemagglutination titre of pooled allantoic fluids was compared with that of pooled fluid from 6 control eggs infected with same amount of virus. Mean differences shown are based on at least 2 experiments.

Group A 

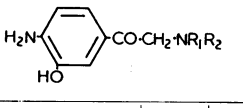
No.	—NR_1R_2	M. Conc. 4×10^{-5}	2×10^{-5}	1×10^{-5}	0.5×10^{-5}
A-1	—NH_2	1·1	0·9	0·6	0·3
A-2	—NH.CH_3	1·3	0·7	0·5	0·2
A-3	—NH.C_2H_5	1·6	0·7	0·2	0
A-4	—NH.CH_2.$CH(CH_3)_2$	>1·7	1·2	0·7	0·3
A-5	—NH.$C(CH_3)_3$	>1·7	1·6	0·7	0·4
A-6	—NH.$[CH_2]_3$.CH_3	1·9	1·1	0·7	0·2
A-7	—NH.CH(cyclohexyl)	>1·4	1·4	0·5	0·3
A-8	—NH.$CH(CH_3)$.CH_2.CH_2.C_6H_5	1·6	1·6	0·9	0·4

Group F 

F-1	—NH.CH_3	>1·7	>1·7	1·5	0·3
F-2	—$N(CH_3)_2$	>2·0	>2·0	0·82	0·45
F-3	—NH.$CH(CH_3)_2$	>1·7	>1·7	1·4	0·15
F-4	—N(cyclohexyl)	1·5	1·5	0·6	0
F-5	—N(morpholine)	1·6	1·6	0·6	−0·2

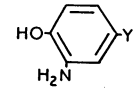
Group O 

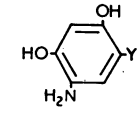
O-1	—NH_2	0·6	0·3	0·2	0
O-2	—NH.$C(CH_3)_3$	2·2	0·7	0·3	0·1

Group P 

P-1	—NH_2	0·6	0·4	0·1	0·1
P-2	—NH.$C(CH_3)_3$	1·0	0·5	0·2	−0·1

Negative results were obtained when the substituted phenyl group of the compounds listed in Table I was replaced by the following groups: *p*-hydroxyphenyl (10), *m*-aminophenyl (4), 2:4-dihydroxyphenyl (7), 3:4-dihydroxyphenyl (7), 2-amino-4-hydroxyphenyl (4), 4-hydroxy-3-nitrophenyl (13). The numerals in parentheses denote the number of primary, secondary and tertiary ω-aminoacetophenones tested in each group. Methylation of the hydroxyl group or acetylation of the amino group in the aromatic nucleus of compounds A-2 and A-5 also caused a loss of antiviral activity. The active compounds of groups A, O, and P had a primary or secondary amino group in their aliphatic chain. Compounds of these types that had a tertiary amino group or an acetylated amino (acetamido) group proved to be inactive. On the other hand compounds of group F with a tertiary amino group in the aliphatic chain were highly active. The specificity of the structure of the aliphatic chain for the antiviral activity of ω-aminoacetophenones with appropriate substituents in the aromatic nucleus was illustrated by the negative results obtained with other compounds of the general types I and II below. In these formulae the symbols R_1 and R_2

I 

II 

I Y= −H, $-CH_3$ −OH, $-NH_2$,
−CHO, $-CO_2H$, $-SO_3H$
$-CO.CH_2R_1$(6), $-CO.CH(CH_3).NR_1R_2$(8)
$-CO.CH_2.CH_2.NR_1R_2$(6), $-CO.CH_2OR_1$(4)

II Y= −H, $-CO_2H$,
$-CO.CH_2R_1$(2), $-CO.CH_2.CH_2.NR_1R_2$(2)

represent a hydrogen atom or an alkyl, *cyclo*alkyl, arylalkyl or saturated nitrogen-containing heterocyclic ring. The numerals in parthentheses give the number of compounds tested in each series. The compounds where Y represents a hydrogen atom or a methyl, hydroxyl or amino group were highly toxic for the chick embryo. Doses of about $1/8\times10^{-5}$ mole/egg were lethal. The remaining compounds, all having a strong electron attracting group at Y, had an LD50 $\geqslant 4\times10^{-5}$ mole/egg.

The apparent antiviral effects shown by the compounds listed in Table I are not due to interference of these compounds with determinations of the haemagglutinin titres of the viral suspensions harvested from the eggs. There was no significant difference between the titres of influenza FM_1 suspensions diluted with equal volumes of 0.02 M solutions of A-2 or A-5 and suspensions diluted

with an equal volume of saline. Breakdown products of A-2 or A-5 formed during the incubation period also did not disturb the determination of the titres. Table I suggests that the strength of the antiviral action in the A-series becomes greater with an increasing number of carbon atoms attached to the secondary nitrogen atom of the aliphatic chain. A comparison of the results obtained with compounds of groups A and F suggests that the latter compounds are more active than corresponding numbers of group A. This suggestion was confirmed by calculation of the relative potencies of two compounds by means of a (4×4) factorial scheme (Philippe, 1955). Taking the activity of A-2 as unity the 95% confidence limits of the calculated activity of A-5 are 2.0 to 3.9; of A-7, 2.3 to 4.6; and of F-3, 2.5 to 4.8.

A determination of the negative log ID50 of the allantoic fluids harvested from eggs incubated 48 hr. after infection with FM_1 virus and injection of the compounds confirmed the conclusions about activity based on measurement of the log haemagglutinin titre (Table II).

For closer investigation of antiviral activity most experiments were made with A-5 and F-3 as representatives of the inhibiting compounds. Table III shows that administering A-5 as early as 16 hr. before or as late as 24 hr. after inoculation of the seed virus still caused a significant, though smaller, decrease of virus growth.

When the seed virus was less diluted and consequently the amount of virus injected into the allantoic fluid of the eggs was much larger than the amount inoculated in the standard procedure, the antiviral action of A-5 was much less pronounced. A dose of $4\text{–}10^{-5}$ mole A-5 showed no antiviral effect when an undiluted seed virus was used. Significant antiviral effects were observed with hundred-fold or higher dilutions of the seed virus.

A determination of the change in haemagglutinin concentration with time showed that increasing the dose of A-5 administered 1 hr. before inoculation of the virus delayed the moment at which virus growth became detectable in the allantoic fluid. Once detectable, the velocity at which the haemagglutinin subsequently increased appeared not to differ very much from that in control eggs (Fig. 1, curves *a*, *b*, *c*, and *d*). Determination of the negative log ID50 of the allantoic fluids at different times gave the same result (Fig. 2). A comparison of these curves with curves *f* and *g* in Fig. 1 suggests that the main effect of A-5 was an inactivation of a considerable part (99.9 to 99.99%) of the seed virus before attachment to susceptible cells. This suggestion is supported by the curve *e* in Fig. 1. Administration of A-5 24 hr. after the injection of the seed virus caused a subsequent rapid drop of the

TABLE II

EFFECT OF SUBSTANCES ON VIRUS GROWTH MEASURED BY THE NEGATIVE LOG ID50 AND LOG HAEMAGGLUTININ TITRE

Determinations made on a pool of allantoic fluid harvested after 48 hr. of incubation at 36° from eggs injected with 4×10^{-5} mole of a compound dissolved in 0·2 ml. saline, and 1 hr. later with 0·1 ml. of influenza virus FM_1, both being injected into allantoic cavity. Control eggs received 0·1 ml. of virus and 0·2 ml. saline.

Comp. See Table I	Log Titre Haemagglutinin	Difference from Control	Neg. Log ID50	Difference from Control
Control ..	3·0		8·5±0·3	
A-2	1·8	1·2	7·4±0·4	1·0±0·4
A-5	1·2	1·8	5·4±0·4	3·0±0·5
F-3	<1·2	>1·8	3·5±0·3	5·0±0·4

TABLE III

EFFECT OF INTERVAL BETWEEN INOCULATION OF VIRUS AND ADMINISTRATION OF A-5 ON CONCENTRATION OF HAEMAGGLUTININ IN ALLANTOIC FLUID OF EGGS MEASURED 48 HR. AFTER VIRUS INOCULATION (AT ZERO TIME) AND INJECTION OF 4×10^{-5} MOLE A-5

Interval (hr.)	−16	−2	−1	0	+1	+5	+24	Control
Log haemagglutinin titre ..	2·15	1·3	1·3	1·3	1·5	1·8	2·0	2·8

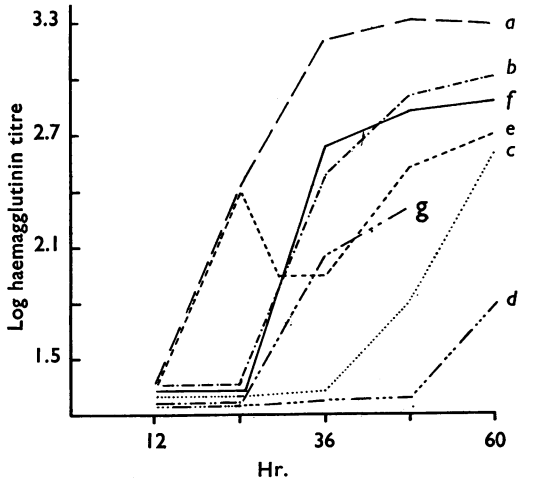


FIG. 1.—Changes with time of log haemagglutinin titre of pooled allantoic fluids from six eggs incubated at 36° after injection into allantoic cavity, in (*a*) to (*e*) inclusive, of 0·1 ml. of 10^{-4} standard virus suspension, and: *a*, no other injection; *b*, 10^{-5} mole A-5/egg 1 hr. before; *c*, 2×10^{-5} mole A-5/egg 1 hr. before; *d*, 4×10^{-5} mole A-5/egg 1 hr. before; *e*, $1{\cdot}5 \times 10^{-5}$ mole A-5 24 hr. later; *f*, 0·1 ml. of 10^{-7} standard virus suspension only; *g*, 0·1 ml. of 10^{-8} standard virus suspension only.

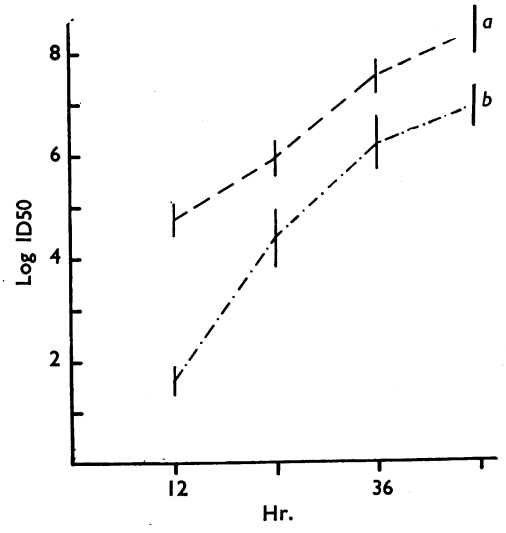


FIG. 2.—*a*, Increase of infecting power (negative log ID50) of pooled allantoic fluids from six eggs after injection of 0·1 ml. of 10^4 dilution of seed virus and 0·2 ml. of 0·9% saline. *b*, Increase of infecting power when 3×10^{-5} mole A-5/egg injected instead of saline. Incubated at 36°. Range marks indicate standard deviations.

haemagglutinin concentration. Further, this experiment indicated that the effective concentration of the inhibitor decreased rapidly.

An investigation of the direct action of the derivatives of acetophenone on influenza FM_1 was made *in vitro* (Tables IV and V). Compounds were added to virus-containing allantoic fluids in concentrations about equal to those used in the antiviral egg test. Incubation of these mixtures at 37° decreased their haemagglutinating and infecting power. The direct action on infecting power appeared to be more intense than the direct action on haemagglutinating power. At 4° or at 15°, the virucidal and the haemagglutinin destroying action was less powerful. The virucidal action of the derivatives of acetophenone appeared to be much weaker than that of formaldehyde, which has no antiviral effect in the egg test. Several derivatives of ω-aminoacetophenone having no antiviral effect in the egg test appear to have a virucidal and a haemagglutinin destroying action *in vitro*. Some examples are shown in Table IV. The structural relationships observed for the egg test therefore are not valid for the *in vitro* tests.

The importance of the *o*- and *p*-aminophenol structure for antiviral activity in the egg test led us to believe that this activity might be connected with the easy oxidizability of these compounds. Therefore we investigated whether the administration of reducing substances at the same time would suppress the antiviral action. Separate experiments had shown that ascorbic acid and sodium bisulphite protect a neutral solution of A-5 from oxidation by air. These reducing substances influence neither the results of the egg test nor the action *in vitro*. (±)-Cysteine, (±)-methionine, (−)-tryptophan, (−)-histidine, nicotinic acid, riboflavine, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid and 3-amino-4-hydroxy-

TABLE IV

IN VITRO ACTION OF ω-AMINOACETOPHENONES AND ANALOGUES ON THE INFECTIVITY OF INFLUENZA FM_1 VIRUS SUSPENDED IN ALLANTOIC FLUID

I. 4×10^{-3} M solutions of compounds in infective allantoic fluid incubated at 37°. The values given are the negative log of ID50. II. 4×10^{-3} M solutions in infective allantoic fluids incubated for 48 hr. at 37°. Infectivity is expressed as fraction of 6 eggs apparently infected by injection of 0·2 ml. of the incubation mixture. III. As for II, but incubated at 4°.

	Comp.	Incubation Time (hr.) 0	5	24	48	Result in Egg Test
I	A-2	8·5±0·4	7·1±0·4	0·6		+
	A-5	8·0±0·3	5·6±0·3	<0·5		+
	F-3	7·7±0·5	3·5±0·4	≤0·5		+
	ω-*tert.*-Butylamino-*p*-hydroxyacetophenone	8·4±0·3	6·4±0·3	1·2±0·2		−
	4-Hydroxy-ω-methylamino-3-nitroacetophenone	8·7±0·3	6·6±0·3	≤0·5		−
	3 : 4-Dihydroxy-ω-*iso*propylaminoacetophenone	8·6±0·3	5·5±0·3	≤0·5		−
	Phenol	7·7	7·2	6·0	3·4	−
	Formaldehyde	5·5	−0·5	≤−0·5		−
	Control	8·5±0·4	8·2±0·4	5·6±0·3		
II	A-5	−	5/6	0/6	0/6	+
	F-3	−	6/6	2/6	0/6	+
	3-Acetamido-4-hydroxy-ω-*iso*propylaminoacetophenone	−	5/6	1/6	0/6	−
	3-Amino-4-hydroxy-ω-piperidinopropiophenone	−	4/4	5/5	0/6	−
	Control	−	6/6	6/6	0/6	−
III	A-5	−	5/5	5/5	−	+
	F-3	−	6/6	4/4	−	+
	Control	−	6/6	6/6	−	−

TABLE V

IN VITRO ACTION OF ω-AMINOACETOPHENONES ON HAEMAGGLUTINATING POWER OF SUSPENSIONS OF FM_1 VIRUS IN ALLANTOIC FLUIDS

Compounds (4×10^{-3}M) dissolved in allantoic fluids containing virus.

Comp.	Incubation Temp.	Log Haemagglutinin Titre After 0 Hr.	24 Hr.	72 Hr.	120 Hr.	168 Hr.
Control	37	3·0	2·8	2·8	2·9	2·9
A-5	37	2·9	2·6	2·4	2·0	1·6
A-5	15	3·0	2·9	2·8	2·8	2·6
A-5	4	2·9	3·0	2·8	2·9	2·7
F-4	37	2·9	2·0	1·6	1·2	1·2
Phenol	37	2·4	2·7	2·8	2·8	2·7
Formaldehyde	37	2·4	2·7	2·7	2·6	2·7

benzoic acid were also tried as antagonists of the antiviral action of A-2 or A-5. They failed to show any effect.

Tests in Other Host Systems

Injection of 4×10^{-5} mole of F-3 or of 2×10^{-5} mole of F-4 into the yolk sac of the embryonated egg, followed 4 hr. later by infection of the yolk sac with 0.2 ml. of virus-containing allantoic fluid with a haemagglutinin titre $\leqslant 2.6$ diluted a thousand times failed to show any effect on the mean survival time of the embryo.

In a test with Swiss albino mice, daily subcutaneous injection of 50 mg./kg. of A-5 failed to prevent infection, and also failed to protect the animals against the consequences of intranasal infection with $3 \times$ LD50 influenza FM_1 virus.

Discussion

Inhibition of multiplication of influenza virus FM_1 in embryonated eggs by derivatives of ω-aminoacetophenone must be ascribed to their virucidal action. On the other hand substances with chemical structures closely related to those of the compounds active *in ovo* are equally virucidal *in vitro*, but have no effect on virus growth in the egg.

These different effects cannot be explained. During the initial period after administration of drug and virus to the egg, the concentration of virus in the allantoic fluid is very low and nothing is known about possible differences in virucidal action at these low concentrations. The experiments *in vitro* indicate that the virucidal action of the derivatives of acetophenone is not very effective. Therefore it seems reasonable to suggest that, in view of the long incubation period of 48 hr., differences in the rate at which these virucidal structures disappear from the allantoic fluid will play an important rôle.

In the molecules of the substances active in the egg test two important structural elements can be distinguished. These are the structures of *o*- or *p*-aminophenol and of ω-aminoacetophenone. Every change in each of these two structural elements reduced activity in the egg test. Other investigations have already shown that derivatives of phenol such as hydroquinone, *o*-aminophenol and *p*-aminophenol are virucidal for the influenza viruses (Kuroya, 1953; Hannoun, 1953; Groupé, Engle, Gaffney, and Hannaker, 1956; Spizizen, 1943; Kramer, Robbins and Smith, 1955). Virucidal activity has also been reported for acetophenone and some of its derivatives (Hartmann, 1954). These derivatives of phenol and of acetophenone, however, did not inhibit the development of influenza virus haemagglutinin in the allantoic fluid of infected embryonated eggs (Takemoto, Robbins, and Smith, 1954). Therefore it is interesting that the combination of these structural features in our compounds produced activity *in ovo*.

The inactivity of ω-aminoacetophenones with other substituents in the aromatic nucleus and the activity of those containing the *o*- and *p*-aminophenol structures irrespective of the position of the amino and hydroxyl group relative to the side chain indicate that specific properties of these structures are responsible for the antiviral action in the egg test. Both *o*- and *p*-aminophenols are easily oxidized. Preliminary experiments failed to show that the biological effects are connected with this property.

The introduction of a ketonic side-chain into the molecule of *o*- and *p*-aminophenol greatly reduces the toxicity of these substances for the chick embryo. The same effect is caused by other electron attracting groups. Changes of this kind also reduce the bactericidal and virucidal potency of derivatives of phenol (Takemoto *et al.*, 1954; Kramer *et al.*, 1955; Barber and Haslewood, 1945). The high specificity apparent from the investigation of several side-chain structures may be due in part to a differential reduction of the toxicities for host and virus.

These considerations, however, do not explain the special importance of the primary or secondary ω-amino group, present in representatives of groups A, O, and P. Chemically, primary and secondary α-aminoketones are unique in that they might form dihydropyrazines by an intramolecular condensation in the weakly basic allantoic fluid. It may be relevant that the carcinolytic (Lutz, 1955) and regeneration-inhibiting activity (Lehmann, Bretscher, Kühne, Sorkin, and Erne, 1950) of α-aminoketones has been ascribed to the formation of dihydropyrazines (Hinderling, Prijs, and Erlenmeyer, 1956). ω-Aminoacetophenones with a tertiary amino group in the aliphatic chain cannot be involved in this intramolecular condensation. The activity in the egg test of tertiary amino compounds of group F therefore makes the significance of this chemical possibility questionable unless one assumes that in the case of group F the substitution in the aromatic nucleus, which has the configuration of both *o*- and *p*-aminophenol is the determining factor. The greater antiviral action of compounds of group F than that of corresponding compounds of group A illustrates this possibility.

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