On the Teratogenic Interaction of Sulfanilamide and 3-Acetylpyridine in Chick Development [1]

WALTER LANDAUER AND ELLEN M. CLARK
Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Connecticut

The metabolic interactions between teratogens, whether leading to synergistic, modified or reduced activity, are of great theoretical and practical interest. In continuation of earlier work (Landauer and Clark, '62a–c), we have now made new inquiries into such events and their results will be presented in what follows.

Two niacin analogs and antagonists, 6-aminonicotinamide (6-AN) and 3-actylpyridine (3-AP), are known to produce potent effects on chick development. These effects are quite different for the two teratogens, viz., treatment with 6-AN leading to dwarfing, micromelia and parrot beak, the administration of 3-AP resulting in muscular hypoplasia and — more rarely — a shortening of the upper beak (Landauer, '57). In subsequent experiments it was found that, when administered in proper ratio, the injection of 3-AP added to that of 6-AN will prevent, altogether or nearly completely, the occurrence of those malformations which are the typical result of treatment with 6-AN, but that, on the contrary, the defects due to 3-AP tend to become exaggerated in the presence of 6-AN (Landauer and Clark, '62b).

Our new experiments were designed to shed light on a number of obscure points, to wit: Is the remarkable interaction between 6-AN and 3-AP, to which reference has just been made, related to the fact that both teratogens are analogs of nicotinic acid amide, or would two chemically unrelated compounds, with comparable teratogenic activities, produce similar results? What, if any, effect does varying the developmental stage at treatment have on the results? Was the great disparity in teratogenic effectiveness of 6-AN and 3-AP, expressed in molar terms, related to the outcome of our tests? Was *in ovo* conversion of 3-AP to nicotinamide likely to have played a role in protecting embryos against the consequences of administered 6-AN? Finally, it seemed of interest to secure information concerning the effects of a micromelia-inducing compound, other than 6-AN, on the teratogenic activity of 3-AP.

Since 3-acetylpyridine is the only teratogen now known which produces in chick development a high incidence of muscular hypoplasia, and since a variety of micromelia-inducing compounds are readily available, we decided to use 3-AP in combination with a teratogen which has effects similar to those of 6-AN and which, while responding to supplements of nicotinamide, is not chemically related to it. Among the several substances answering to these specifications, we chose sulfanilamide (SA) because of its relatively low toxicity and high micromelia-inducing activity.

The effect of sulfanilamide and other sulfonamides on development of chicken embryos was first described by Ancel (Ancel and Lallemand, '42; Ancel, '45) who found in extensive experiments that, dropped onto the blastoderm of embryos after 48 hours of incubation, SA led to a syndrome of micromelia and parrot beak (i.e. a shortening of the mandible and a down-curving of the maxilla). Zwilling and DeBell ('50) confirmed and greatly extended Ancel's observations in experiments in which solutions of SA were injected into the yolk sac at various stages and in several dosages. They found *inter alia* that supplementary para-aminobenzoic acid had no alleviatory effect, but that micromelia and parrot beak did not occur when nicotinamide was added to the administration of SA.

[1] Scientific contribution no. 88, Agricultural Experiment Station and no. 106 of the Institute of Cellular Biology, University of Connecticut.

In our present experiments we used essentially the same material and techniques that had served us earlier. An initial test at 96 hours of incubation (data of table 1) was done with eggs of White Leghorn *hens;* in the three subsequent tests (data of tables 2 to 4) the eggs came from White Leghorn *pullets*. The differences in age or in genetic make-up between the two stocks presumably account for dissimilarities in response to 3-AP at 96 hours, dissimilarities which did not affect any of our principal conclusions. The dosages of teratogens were kept constant throughout the experiments, viz. 1 mg/egg (5.81 μM) SA and 0.5 mg/egg (4.13 μM) 3-AP. Solutions of the two teratogens were made up and, with one exception to be noted below, injected separately, the injection of SA always preceding that of 3-AP.

RESULTS

We carried out four series of tests. In the first of these, all injections were done at the 96-hours stage. In subsequent tests the embryos were treated in different developmental stages and the administration of 3-AP followed that of SA by 24 hours, viz, 48 and 72, 72 and 96, 96 and 120 hours, respectively. The results are recorded in tables 1 to 4. In the initial experiment in which all injections were given at 96 hours (table 1), the combination of SA and 3-AP was tested in two different ways; in one of these, the 3-AP was dissolved in the solution of SA, in the other, the two teratogens were injected separately; since the two tests produced nearly identical results, the data were pooled.

Examining the outcome of the experiment at 96 hours, it can be seen that among 200 embryos surviving the thirteenth day of incubation, following the injection of 1 mg/egg SA, 53.5% showed abnormalities of the long bones of the legs and 43% had a parrot beak. Among the 107 embryos with micromelia of one degree or another, 58.9% were recorded as severely micromelic. No signs whatever of micromelia or parrot beak occurred when treatment with 1 mg SA was combined with or immediately followed by administration of 0.5 mg 3-AP. A very different situation existed with regard to the syndrome of muscular hypoplasia, shortened upper beak and twisted neck (the last an expression of muscular abnormality encountered in the more extreme deviants from normal), occurring after the administration of 3-AP. Here was found in the presence of SA a great exaggeration in the incidence of muscular hypoplasia and twisting of the neck (P in both cases being $<< 0.0001$). The frequency of a shortened upper beak was also raised, but not significantly so.

TABLE 1

The effect of 1 mg sulfanilamide and 0.5 mg 3-acetylpyridine alone or in combination when injected into the yolk sac of White Leghorn eggs after 96 hours of incubation. December 1963

	SA	SA + 3-AP	3-AP
Number treated	237	460	233
Mortality in %			
to end sixth day	11.0	13.5	9.0
7–13	4.6	13.5	11.2
14–17	0.4	4.6	3.4
18–22	49.8	67.2	69.1
Hatched, %	34.2	1.3	7.3
Number surviving thirteenth day	200	336	186
Normal, %	40.0	10.4	29.0
Micromelia,[1] %	53.5	—	—
Parrot beak, %	43.0	—	0.5
Muscular hypoplasia, %	—	83.0	65.6
Short upper beak, %	—	7.1	4.8
Twisted neck, %	—	16.7	3.2
Miscellaneous defects, %	3.0	1.5	2.2

[1] All cases with a recognizable shortening of the legs, including some in which bending of the tibia appeared to be the only abnormality.

Turning to the experiments in which treatment with SA at 48, 72 and 96 hours was followed after an interval of 24 hours by injection of 3-AP, it can be seen that in the control groups receiving only SA, the incidence of micromelia rose significantly ($P < 0.001$) from 48 to 96 hours of treatment. It must be noted, however, that there was virtually no change (26.9 vs. 25.7%) in the frequency with which *high grades* of the condition occurred, the great majority of the additional cases of long bone involvement having a bent tibia as the only recorded abnormality. This situation is reflected in a significant *decrease* ($P < 0.01$), from the 48 to the 96 hours stage, in the proportion of the most *severe* grade of shortening among all recorded instances of micromelia. The rise in overall incidence of micromelia between 48 and 96 hours of treatment is paralleled by a less striking increase in the frequency with which parrot beak occurred (P between 0.03 and 0.04). In the experiments with combined administration of SA and 3-AP a few cases of micromelia (exclusively low grade) and parrot beak persisted in the experiment conducted at 48 and 72 hours (table 2), but in the two subsequent experiments (tables 3 and 4) treatment with SA produced no micromelia in the presence of 3-AP, and the incidence of parrot beak remained below 1%.

In examining the effect which the injection of 0.5 mg 3-AP has in different developmental stages, we find that the over-all incidence of muscular hypoplasia was 60.5, 47.4 and 36.1% following treatment at 72, 96 and 120 hours, respectively. Moreover, this decline occurred chiefly at the expense of those cases that had been recorded as "pronounced," the corresponding incidence figures being 46.1, 28.1 and 21.6% (for the difference between 72 and 120 hours P is < 0.01). The sharpest drop in involvement of muscular development occurred between 72 and 96 hours and the same is true for shortness of the upper beak. The differences in incidence of muscular hypoplasia (all grades) and of shortened upper beak, either 72 vs. 96 or 72 vs. 120 hours, are all highly significant ($P << 0.0001$).

Finally, there are the remarkable effects which the presence of SA has on teratogenic activity of 3-AP. When SA was injected at 48 hours, followed by 3-AP at 72 hours, the incidence of muscular hypoplasia, compared to treatment at 72 hours with only 3-AP, was reduced from 60.5 to 19.7% (table 2, $P << 0.0001$). Moreover none of the embryos in the group treated with both teratogens showed a degree of muscular hypoplasia as extreme as that of many of the specimens in the 3-AP control group. The incidence of shortened

TABLE 2

The effect of 1 mg sulfanilamide and 0.5 mg 3-acetylpyridine alone or in combination on embryos of White Leghorn fowl. SA injected at 48 hours, 3-AP at 72 hours of incubation. December 1963

	SA	SA + 3-AP	3-AP
Number treated	298	300	298
Mortality in %			
1–6	11.1	16.0	14.4
7–13	1.7	6.3	9.1
14–17	0.7	2.0	4.7
18–22	52.3	65.0	66.1
Hatched, %	34.2	10.7	5.7
Number surviving thirteenth day	260	233	228
Normal, %	49.6	70.4	32.9
Micromelia,[1] %	46.2	4.7	—
Parrot beak,%	38.1	3.0	—
Muscular hypoplasia, %	—	19.7[2]	60.5
Short upper beak, %	—	4.7	21.5
Twisted neck, %	—	—	9.2
Miscellaneous defects, %	0.8	3.4	3.9

[1] All degrees of shortening of legs in SA-group, including some in which bending of tibia or reduction in length of tarsometatarsus was the only recognizable symptom, but only these minor expressions found in SA + 3-AP group.

[2] None of these were as extreme as many in group treated with 3-AP.

upper beak was reduced in a similarly drastic manner, 21.5 vs. 4.7% ($P << 0.0001$).

The sparing or protective effect which the presence of SA had on teratogenic activity of 3-AP at the 48–72 hours stages, changed in subsequent development to a striking form of synergism (tables 3 and 4). Injection of SA at 72 hours and of 3-AP at 96 hours produced an incidence of 65.7% muscular hypoplasia compared with 47.4% in the group receiving only 3-AP at 96 hours ($P << 0.0001$). The significance of this difference is underlined by the fact that the extent of hypoplasia tended to be much more extreme in the group which received the combination treatment than among the 3-AP controls. The occurrence of a shortened upper beak showed a similar trend, but the incidence was too low to establish significance. The last group (table 4) gives the results of treatment with SA at 96 hours, followed

TABLE 3

The effect of 1 mg sulfanilamide and 0.5 mg 3-acetylpyridine alone or in combination on embryos of White Leghorn fowl. SA injected at 72 hours, 3-AP at 96 hours of incubation. December 1963

	SA	SA + 3-AP	3-AP
Number treated	304	304	300
Mortality in %			
to end sixth day	7.6	13.2	11.3
7–13	3.9	9.2	4.3
14–17	1.3	2.3	1.3
18–22	61.2	73.0	69.7
Hatched, %	26.0	2.3	13.3
Number surviving thirteenth day	260	236	253
Normal, %	44.2	36.0	51.8
Micromelia,[1] %	55.4	—	—
Parrot beak, %	47.3	0.8	—
Muscular hypoplasia, %	—	65.7 [2]	47.4
Short upper beak, %	—	3.8	2.4
Twisted neck, %	—	3.4	2.4
Miscellaneous defects, %	—	0.4	1.6

[1] All grades.
[2] The majority more extreme than in group treated with 3-AP alone.

TABLE 4

The effect of 1 mg sulfanilamide and 0.5 mg 3-acetylpyridine alone or in combination on embryos of White Leghorn fowl. SA injected at 96 hours, 3-AP at 120 hours of incubation. December 1963

	SA	SA + 3-AP	3-AP
Treated	292	291	296
Mortality in %			
to end sixth day	6.8	10.3	2.7
7–13	1.4	2.7	1.7
14–17	1.7	0.7	0.3
18–22	49.3	84.9	65.9
Hatched, %	40.8	1.4	29.4
Number surviving thirteenth day	268	253	283
Normal, %	36.6	9.1	59.7
Micromelia,[1] %	61.6	—	—
Parrot beak, %	47.4	0.8	4.6
Muscular hypoplasia, %	—	90.1	36.1
Short upper beak, %	—	54.9	4.9
Twisted neck,%	—	62.8	5.7
Miscellaneous defects, %	1.1	—	1.8

[1] All grades.

by injection of 3-AP at 120 hours and of controls which received only 3-AP at 120 hours. Here we find an extreme exaggeration of the 3-AP-induced symptoms. Incidence of muscular hypoplasia was 90.1 instead of 36.1%, the frequency of twisted neck rose from 5.7 to 62.8%, and 54.9 compared to 4.9% of the embryos had a shortened upper beak (for all these differences ($P << 0.0001$). The most dramatic demonstration of this exaggeration was provided by comparing the incidence in the two groups of simultaneous presence in the same embryos of muscular hypoplasia, twisting of the neck and shortening of the upper beak, a triad that is always associated with a great reduction in body size. The injection of 3-AP at 120 hours produced among survivors of the thirteenth day 1.8% of this syndrome, but its incidence rose to 45.8% when treatment with 3-AP was preceded by administration of SA!

In contrast to the decline, between 72 and 120 hours of treatment, in incidence of muscular hypoplasia and of shortening of the upper beak called forth by treatment with 3-AP alone, we found, during the same period, a great increase in the frequency of both types of defect if treatment with 3-AP was preceded by the injection of SA (differences with a $P << 0.0001$). Among all cases of muscular hypoplasia, observed after the combined treatment, the percentage of those recorded as "slight" was 69.6% after the injection of 3-AP at 72 hours, dropped to 34.8% for the experiment at 96 hours, and was a mere 10.5% when SA administration was followed by injection of 3-AP at 120 hours; the reciprocal values illustrate the rising severity of failure in normal muscular development.

DISCUSSION

Our observations on the effects of sulfanilamide on chick development agree in nearly every detail with the findings reported by Ancel and by Zwilling and DeBell. We found, as did the latter authors, that with a constant dose of SA a significantly higher proportion of embryos showed severe forms of micromelia following treatment in early as compared to late stages (48 vs. 96 or 120 hours of incubation); but in our material the over-all incidence of involvement of the long bones of the legs rose from 48 to 96 hours of administration, the symptoms in an increasing proportion of cases being limited to a bending of the tibia shaft.

The consequences of supplementary administration of 3-AP on incidence of SA-induced micromelia were dramatic. When injection of SA at the 48-hours stage was followed 24 hours later by 3-AP, the frequency of micromelia among the treated embryos was about 10% of that found after unsupplemented SA-treatment, and no cases at all of micromelia were encountered when the injection of SA at either 72 or 96 hours of incubation was followed 24 hours later by the administration of 3-AP or when SA and 3-AP were both given at 96 hours. The protective effectiveness of 3-AP against the interference of SA in development was similarly drastic in regard to the parrot-beak symptom.

It is clear then that the protection which, as reported by us earlier, 3-acetylpyridine furnishes against the teratogenic activity of 6-aminonicotinamide is similarly found in combination with sulfanilamide. Indeed, a comparison based on molar ratios shows that the protective efficiency was much the greater in our present data. In our former experiments we had used 76 to 153 times as many millimoles of 3-AP as of 6-AN to obtain complete protection; the corresponding proportion of 3-AP to SA in the new tests was 0.71! This surprisingly low amount of 3-AP required for protection against the teratogenic activity of SA raised the question whether the known metabolic function of 3-AP suffice as an explanation. Kaplan, Ciotti and Stolzenbach ('56) concluded from their tests that 3-AP·AD had approximately half the metabolic usefulness of NAD. If a similar ratio applies to our material, then a far smaller amount (about 5%) of nicotinamide than had been employed heretofore, should bring about protection against the injection of SA. This problem was put to the test by comparing the results of injecting 1 mg SA alone or in combination with 250 γ nicotinamide, a ratio of 0.35 millimoles nicotinamide to one of sulfanilamide. The results were clear: This small amount of

nicotinamide sufficed to forestall all morphogenetic damage from injected sulfanilmide. Hence it seems safe to conclude that the protective value of 3-AP, in our experiments, was due to the metabolic activity of 3-AP substituted pyridine nucleotide.

It is known from the work of Zwilling and DeBell that the teratogenic consequences of injected sulfanilamide can still be prevented if nicotinamide is given several days later. Our findings demonstrate a similar effectiveness of 3-AP 24 hours after treatment with SA. We know, secondly, that injected 3-AP does not interfere with growth in length of the long bones. Finally, we have established that the amounts of 3-AP which are required to prevent or counterbalance the micromelia-inducing activity of sulfanilamide agree well with the demonstrated level of metabolic usefulness of 3-AP·AD. These facts leave little doubt but that the role of 3-AP as protector against sulfanilamide-induced micromelia is exercised by its effects on hydrogen transport mechanisms. This may or may not imply that in the presence of 3-AP the sulfanilamide-sensitive cells become "sites of loss" in Veldstra's sense (Veldstra, '56).

The second part of our discussion relates to the influence which sulfanilamide has on the teratogenic activity of 3-acetylpyridine. The data from tests in which comparable groups were given 3-AP at 72, 96 and 120 hours, respectively, show that with a constant dose (which, of course, is sharply dropping in proportion to embryo weight), the response to 3-AP — measured in incidence of muscular hypoplasia and shortening of the upper beak — declines steeply from 72 to 96 hours (for both $P << 0.0001$). There is a further decrease in incidence of muscular involvement between 96 and 120 hours and a comparison of the records for the 72 and 120-hours groups shows that the data for the latter experiment include a significantly higher proportion of affected embryos in which the hypoplasia was diagnosed as "slight." The high incidence of a shortened upper beak which 3-AP produced at 72 hours is especially noteworthy.

Turning now to the combined administration of SA and 3-AP, we found remarkable differences in response depending on time of treatment. When SA was injected at 48 hours, followed by 3-AP at 72 hours, the occurrence of muscular hypoplasia was only about one third that produced by 3-AP alone (the deviation from normal was, moreover, on the average less pronounced) and the incidence of shortening of the upper beak was reduced to less than one quarter (table 2). These differences were highly significant (for both $P << 0.0001$). When, however, SA was administered at either 72 or 96 hours, followed after 24 hours by 3-AP, or when both drugs were given at 96 hours, there was a great exaggeration in incidence of the most typical consequence of 3-AP treatment, viz., muscular hypoplasia (tables 1, 3 and 4). This synergistic effect was particularly striking when SA was injected at 96 hours and 3-AP at 120 hours (table 4); in this test, but not in those performed on younger embryos, there also occurred a dramatic rise in incidence of shortening of the maxilla (54.9 vs. 4.9% !).

This Janus-faced behavior shown by the 3-acetylpyridine-symptoms in the presence of sulfanilamide defies at present an entirely satisfactory explanation. As stated earlier, it was shown by the studies of Zwilling and DeBell that the teratogenic effects of sulfanilamide can be prevented until relatively late stages of development by supplementary nicotinamide. If it is assumed that in these stages, and as far as muscle development is concerned, sulfanilamide interferes with functions of pyridine nucleotides which are different from those attacked by 3-acetylpyridine, functions which in the absence of SA help to resist the damage caused by 3-AP, then the exaggerations which we found in late stages and after combined treatment become understandable. We encountered significant exaggeration in incidence of muscular hypoplasia (and shortened maxilla) also when injection of 0.5 mg/egg 3-AP at 96 hours was combined with 5 mg/egg Orinase, i.e. 1-butyl-3(p-tolylsulfonyl) urea, an amount of Orinase which, given by itself, is non-teratogenic.

The exaggeration in incidence of a shortened upper beak, found in the experiments performed at 96 and 120 hours, may have a similar basis as that just discussed for muscular hypoplasia. The

maxilla defect produced by 3-acetylpyridine occurs also — with low incidence — as a consequence of injecting relatively large amounts of nicotinamide, and the extremely high incidence of a shortened upper beak reported by Zwilling and DeBell in their tests combining the administration of sulfanilamide and nicotinamide demonstrates presumably, with reference to this abnormality, that sulfanilamide is synergistic with nicotinamide as well as with its 3-AP analog.

But what about the sparing effect which the presence of sulfanilamide had in early stages on defects produced by 3-AP (table 2)? The attainment of a threshold (at which SA becomes involved in the embryo's metabolism) may readily explain the emergence of synergism, but will not account for an earlier effect in the opposite direction. Yet such sparing action was also found, and in this instance at the 96-hours stage, when 3-AP and insulin were injected simultaneously (table 5). Since in these observations insulin-induced micromelia might have introduced a source of error in diagnosing muscular hypoplasia, we made a separate calculation for the 253 non-micromelic embryos in the three tests summarized in the table. The result was a similarly significant lowering in involvement of the musculature ($\chi^2 = 17.27$, $P < 0.0001$). The mechanisms by which these sparing effects are achieved, remain to be explored.

Our results suggest strongly that supplementary 3-acetylpyridine prevents the occurrence of sulfanilamide-induced micromelia and parrot beak by becoming incorporated into and functioning as substituted pyridine nucleotide. It seems likely that this role is essentially identical with that by which 3-acetylpyridine forestalls the occurrence of similar symptoms after the administration of 6-aminonicotinamide (Landauer and Clark, '62b).

In our earlier report we suggested that exaggeration of the teratological effects of 3-AP, in the presence of supplementary 6-AN, may have been brought about by competitive attachment of the two niacin analogs to sites critical for muscular development, the chances of normal morphogenesis being reduced by the metabolically-inert nature of 6-AN·AD. An analogous mechanism may play a role in the synergistic effects which combined administration of SA and 3-AP has after the third day of development. It should, in fact, be noted that the mechanisms of protection and exaggeration may similarly involve competition for critical sites, the difference being that in those sites that are sensitive to 6-AN and SA (viz., the primordia of long bones and mandible), 3-AP·AD provides helpful functions as a substituted pyridine nucleotide, whereas damage to the sites sensitive to 3-AP (skeletal musculature and maxilla) is increased by the additional presence of compounds (6-AN or SA) which neutralize their participation in metabolic activity. Responsiveness of the sites clearly varies with their progressing differentiation.

Finally, all our observations indicate that, in this material at any rate, the specific effects of teratogens are not brought about by selective distribution of noxious compounds, but by selective responses of particular parts to them. This seems of particular interest with reference to the phenocopy problem. The three compounds which we have been discussing, viz. 6-aminonicotinamide, sulfanilamide and 3-acetylpyridine, produce well-defined syndromes. The micromelia and parrot-beak condition found after the administration of 6-AN or SA are the typical features of sev-

TABLE 5

Incidence of muscular hypoplasia after injection at 96 hours of 375 or 500 γ 3-acetylpyridine alone or combined with four units insulin. White Leghorn eggs. April and June 1962, January 1963

3–AP alone			3–AP + insulin			
3–AP γ	N	Muscular hypoplasia	N	Muscular hypoplasia	χ^2	P
375	148	32	113	0	25.87	< 0.0001
500	138	50	104	18	10.51	< 0.01
500	164	81	106	32	9.75	< 0.01

eral lethal genes (review in Landauer, '61); the muscular hypoplasia, twisting of the neck, shortened maxilla and edema brought about by 3-AP are the traits which characterize embryos homozygous for the "crooked neck dwarf" lethal mutation (Asmundson, '45; Pun, '54; Herrmann, Clark and Landauer, '63). Isolated morphological features, the developmental modification of one particular trait, may well be brought about by a variety of cellular responses to forces from without and, as a consequence, it may not be easy to decide in a specific instance if the resemblance of an induced condition and a hereditary feature represent more than instances of superficial and essentially uninteresting coincidence. It is much less likely, however, that complex syndromes affecting independent parts and organs, should arise without having important steps in common. For this reason we believe that such complex phenocopies deserve the particular attention of students of developmental genetics.

SUMMARY

Experiments have been conducted in which the individual and combined effects of sulfanilamide and 3-acetylpyridine on development of chicken embryos were compared. The principal results were as follows:

1. With a constant dose of 1 mg/egg sulfanilamide incidence of micromelia and parrot beak rose following treatment between 48 and 96 hours of incubation.

2. When both teratogens were administered at 96 hours, 0.5 mg/egg 3-acetylpyridine sufficed to prevent the appearance of any malformations due to 1 mg/egg sulfanilamide. This was largely true also when treatment with sulfanilamide between 48 and 96 hours was supplemented 24 hours later with injection of 3-acetylpyridine.

3. With a constant dose of 0.5 mg/egg 3-acetylpyridine the incidence and severity of the typical abnormalities brought about by this analog of niacin, viz., muscular hypoplasia and a shortened maxilla, decline following treatment between 72 and 120 hours of incubation.

4. When sulfanilamide was injected at 48 hours and 3-acetylpyridine at 72 hours, the incidence of muscular hypoplasia and shortened maxilla was much reduced in comparison to the administration of 3-acetylpyridine alone at 72 hours. In contrast, all symptoms due to treatment with 3-acetylpyridine were greatly exaggerated when its injection at 96 or 120 hours had been preceded 24 hours earlier by treatment with sulfanilamide or when both teratogens had been given at 96 hours.

5. Tests with supplements of nicotinamide indicate that the protective role of 3-acetylpyridine is mediated through its incorporation into substituted pyridine nucleotides.

6. The synergism of sulfanilamide and 3-acetylpyridine, as far as the symptoms typical for treatment with 3-acetylpyridine are concerned, resembles the similar exaggeration occurring in the presence of 6-aminonicotinamide.

7. It is believed that the symptoms of protection as well as those of synergism, here reported, can be understood on the basis of competition for sites of specific responses and the sensitivity of particular sites to interference with processes of hydrogen transfer.

ACKNOWLEDGMENTS

Our work has enjoyed the financial support of the National Science Foundation and the truly indispensable assistance of Mrs. Melva M. Larner.

LITERATURE CITED

Ancel, P. 1945 L'achondroplasie. Sa réalisation expérimentale — sa pathogénie. Ann. d'Endocrin., *6:* 1–24.

Ancel, P., and S. Lallemand 1942 Sur une malformation du bec et des membres obtenu chez l'embryon de poulet à l'aide de sulfamides. C. R. Soc. Biol., *136:* 255–256.

Asmundson, V. S. 1945 Crooked neck dwarf in the domestic fowl. J. Hered., *36:* 173–176.

Herrmann, H., E. M. Clark and W. Landauer 1963 Muscle development in the crooked neck dwarf mutant and in the acetylpyridine-treated chick embryo. Acta Embryol. Morphol. Exp., *6:* 169–174.

Kaplan, N. O., M. M. Ciotti and F. E. Stolzenbach 1956 Reaction of pyridine nucleotide analogues with dehydrogenases. Jour. Biol. Chem., *221:* 833–844.

Landauer, W. 1957 Niacin antagonists and chick development. J. Exp. Zool., *136:* 509–530.

——— 1961 The hatchability of chicken eggs as influenced by environment and heredity. University of Connecticut Agricultural Experiment Station Monograph 1.

Landauer, W., and E. M. Clark 1962a Teratogenic interaction of insulin and 2-deoxy-D-glucose in chick development. J. Exp. Zool., *151:* 245–252.

——— 1962b The interaction in teratogenic activity of the two niacin analogs 3-acetylpyridine and 6-aminonicotinamide. J. Exp. Zool., *151:* 253–258.

——— 1962c Interaction of insulin and chlorpromazine in teratogenesis. Nature, *198:* 215–216.

Pun, C. F. 1954 The crooked neck dwarf lethal syndrome in the domestic fowl. J. Exp. Zool., *126:* 101–133.

Veldstra, H. 1956 Synergism and potentiation with special reference to the combination of structural analogues. Pharm. Rev., *8:* 339–387.

Zwilling, E., and J. T. DeBell 1950 Micromelia and growth retardation as independent effects of sulfanilamide in chick embryos. J. Exp. Zool., *115:* 59–81.