Progress in the Chick Edema Problem

By Dr. Leo Friedman
Food and Drug Administration

DSW 552800

It has been almost four years since I first became aware of the problem that we know today as "chick edema disease." Because of our activity on this problem, my colleagues and I have had the opportunity to become acquainted with many scientific groups and individuals working in the same area with whom we have enjoyed a fruitful cooperation and pleasurable association. We hope that they feel as kindly toward us as we do to them, but sometimes, I know, they wish as we do that they had never heard of chick edema disease.

This problem has been most difficult and progress frustratingly slow. Despite the meager amount of new information that can be added at this time, especially since the most recent advances were reported by Dr. Artman recently, it is nevertheless worthwhile to review the several aspects of this problem and see its present status in full perspective.

As you recall, during 1957 an epidemic disease caused millions of dollars in losses among broiler flocks throughout a large part of the U.S. After elimination in succession of all other possibilities, attention was focused on the fat ingredient of the feed as the etiologic agent. A series of reports in 1958 from several laboratories described the manifestations of the disease and definitely implicated a toxic fat or a toxic substance in fat as the cause. The characteristic symptoms were droopiness, ruffled feathers, labored breathing and high morbidity and mortality. Autopsy findings revealed hydropericardium, abdominal ascites (water belly), subcutaneous edema, swollen liver, swollen and pale kidneys, etc. In laying hens the toxic fat caused a rapid drop in egg production. Pullets receiving toxic fat during the full growing period did not come into production, and mortality was very high. Hydropericardium, the most common lesion found in young birds, was not found in birds of laying age.

EDITOR'S NOTE: *This report from the annual meeting of the Animal Nutrition Research Council in Washington is the 10th in a series being published in Feedstuffs. The author, Dr. Leo Friedman, is assistant director of research, division of nutrition, Department of Health, Education and Welfare, Food and Drug Administration, Washington.*

substituted for the usual normal fat, there was poor reproduction and lactation performance. The females on the toxic fat ration whelped pups that were dead or weak, and, furthermore, the mothers seemed to have an insufficient milk supply. When the pups were removed before weaning and fed a normal ration the increase in growth was immediate and dramatic. Also, the females on the toxic fat ration tended to lose hair on their backs and shoulders. With the ration containing toxic fat, post-weaning growth tests (6-18 weeks) with five litters of pups demonstrated inferior growth performance using either weight gain or increases in body length as the criterion.

Work With Other Species

In these experiments with other species, fat that had first been proved to be toxic to chicks had been used. In the case of monkeys, a sample of triolein that had produced irreversible toxic symptoms for no apparent reason later was proved toxic to chicks and was the source from which we isolated a highly purified crystalline "chick edema" factor. At the present time, then, "toxic fat" that produces "chick edema disease" has been demonstrated also to produce deleterious effects in rats, guinea pigs, swine, dogs and monkeys. It should be emphasized that the toxic fat undoubtedly contains many other substances that may have effects in these other species. Definitive information in growth without hydropericardium or ascites."

In chicks, "Microscopic examination of the tissues of the test animals revealed lymphocytic foci in the epicardium and myocardium. Edematous fluid separated the myocardial fibers. Edema of the lungs was a frequent observation in the experimental birds.

". . . Blood pressure indicated the test birds had an elevated average mean pressure in the right ventricle of 6 cm. water and 2 cm. water in the vena cava. Electron micrographs of the myocardium revealed shrunken vacuolated mitochondria in the test animals.

"'Toxic fat' produces a reduction in growth rate of experimental animals. This reduction depends on the age of the animal and the level of 'toxic fat' added to the diet. Hydropericardium and ascites are a frequent lesion in the animals receiving from 1.0 to 5.0% 'toxic fat.' When this level was reduced to 0.25% in the diet, reduced testicular development was a more sensitive criterion than hydropericardium, ascites or weight gain for evaluating chronic toxicity.

"The mechanism by which 'toxic fat' induces hydropericardium and ascites appears to be associated with degeneration and edema of the myocardial fibers. These data would tend to eliminate the kidneys, liver and endocrines as the primary cause of edema. The early development of hydropericardium, increased venous pressure, enlarged hearts, mitochondrial changes in the myocardium and generalized edema suggest that the myocardium may be directly inhibited; however, altered capillary permeability has not been excluded. It is believed that cardiac decompensation and increased capillary permeability act together in producing the excessive extravascular fluid collection and the demise of the animal."

The wide range of susceptibility within and among

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These differences in susceptibility and symptoms in different age groups of the same species should be noted. The feeding of toxic fat to other species has not produced such striking results as with young chicks, with the exception possibly of monkeys. However, every species that has been tested has shown evidence of deleterious effects. Very little work has been done with rats. Our very limited experience indicates that they are much more resistant than chicks in short-term feedings, but that when fed in sufficient dosage, extracts of the toxic fat produce definite deleterious effects as shown by growth depression, enlarged and fatty livers, and marked involution of the thymus.

I recall few reports of the effects of toxic fat on swine, but again, in our own limited experience we have seen depressed growth . . . and have demonstrated the presence of toxic factor in the meat of hogs that had been fed toxic fat.

I am indebted to Dr. Wilcke of Ralston Purina for reports of studies on guinea pigs and dogs. Guinea pigs fed 2½% toxic fat stopped growing after six weeks, and death losses occurred after eight weeks. At a level of 4½% toxic fat weight losses occurred after three weeks and deaths after four weeks. Control groups receiving non-toxic fats did not show weight loss or deaths. The only observed pathology at the conclusion of the experiment was congestion of the lungs and mottled livers.

In experiments with three different breeds of dogs, using Purina Dog Chow in which 10% of toxic fat was

ma factor (CEF) in other species must await tests with purified CEF. Triolein-fed monkeys probably received the "purest" source of CEF. However, purified CEF should be administered to monkeys to verify the implication that the severe toxic symptoms observed with triolein were due to CEF.

Relatively little has been done to throw light on the mechanism of the toxic effect. Flick and Gallo in our laboratories have reported that in young chicks showing symptoms of the disease, the intra-cellular water was not changed. Neither were the total blood and plasma volumes altered so that the observed edema was primarily interstitial. Hemoglobin and hematocrit levels were low and blood glucose levels were decreased in advanced stages of the disease. Plasma sodium, potassium and chloride were not affected. In the liver, neutral fat was decreased and phospholipid increased. Preliminary experiments by Flick give some indication of increased membrane permeability, but these must be repeated under much more rigorous conditions.

Dissertation Abstract

A most interesting study in chicks has been reported by J. R. Allen, Jr. Part of the results were presented at the federation meetings in March, 1961, and the complete study is available as the dissertation of J. R. Allen, Jr., University of Wisconsin, 1961. I will quote portions from the dissertation abstract [Dissertation Abstracts 22, [2], 545 (Aug., 1961)]:

"Experiments conducted to determine the effects of 'toxic fat' on mice, pigeons and turkeys demonstrated a

variety of toxic effects that already been noted would make it year logical that some primary of structure and function such as mitochondrion, may be the targe the toxic factor and that the obse differences may be explained by tors such as absorption, specific b ing, transport, detoxication, etc., determine the local concentratio any substance in a specific site.

Another possible mechanism had suggested itself quite early that the toxic factor interferes v the normal regulation of electro and water balance. Selye and St back in 1943, described the pro tion of edema symptoms in chicks certain steroids and the accentua of these symptoms by increasing salt intake of the chicks. Alexa has shown that it is possible to duce hydropericardium in chicks increasing the NaCl in the ration to prevent its occurrence even CEF by eliminating NaCl from diet. Work in our own and other oratories has illustrated beautif the interaction of nutritional fac on the susceptibility of the chick a toxic agent. For example, the ent AOAC bioassay diet for "c edema factor" is probably four ti more sensitive than the assay we used originally, although have approximately the same N content.

A point to remember is that hy pericardium as a symptom of toxi in chicks is not new. In additio NaCl and certain steroid hormo chapter 40 in Biester and Schw on Poisons and Toxins indicates t

(1) Zinc phosphide, used as a

(Continued on page 22)

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denticide produces "various degrees of congestion with the accumulation of some serous fluid in the pericardial sac as well as in the abdominal cavity in some cases."

(2) Alpha naphthyl thiourea, the rodenticide ANTU, shows in poisoned chicks evidence of lung edema and excessive quantity of fluid in the pericardial sac.

(3) Sodium monofluoroacetate, compound 1080, another very effective rodenticide, produces in chicks distention of the pericardial sac with clear straw-colored fluid, in addition to other marked pathological changes on the heart and lungs.

(4) Chlordane. "The primary lesions found in all fatal cases were in the heart. Excessive quantities of fluid were found in the pericardial sacs. . . ."

The weed "corn cockle" and several species of Crotalaria produce seeds which are toxic, and in chicks the toxic symptoms include hydropericardium.

However, in each case other characteristic pathology is usually present, and in no case is the purified toxic principle of the same high order of activity as the toxic substances that have been isolated from toxic fats.

It is well known now that chicks can efficiently utilize large amounts of fat in properly balanced rations. The use of fat as a standard ingredient of poultry feeds grew as the price of fat calories dropped and became competitive with calories derived from corn. The chick edema disease epidemic of 1957 was a totally unexpected consequence of this growing practice. Many of you are familiar with the story of how the toxicity was associated with a fatty by-product of stearic and oleic acid manufacture that had been blended with feed grade fats.

Very large quantities of fatty acids are used industrially in the manufacture of lubricants, rubber, paints, asphalts, roofing, chemicals, and to a much smaller extent in foods. Relatively low grades of fat are split into fatty acids and glycerol at high temperatures and pressures, sometimes with the aid of catalysts. The glycerol is recovered and the fatty acids are distilled under vacuum. The first distillate may be used directly as the highest grade of mixed fatty acids, or it may be separated by a low temperature crystallization process into stearic (saturated) and oleic (unsaturated) fractions. The residue from this distillation is resplit and redistilled. The second distillate yields a lower grade of fatty acids. The residue from the second distillation is usually suitable for use on highways or in rubber manufacturing, but occasionally it is again recycled to obtain a third distillate and another residue. It was this third residue that had been blended with feed grade fats for use in feeds.

Every sample of residue of this type from several manufacturers of fatty acids proved to be rich in chick edema toxicity. Our first impression, therefore, was that the toxic factor was produced during the splitting and

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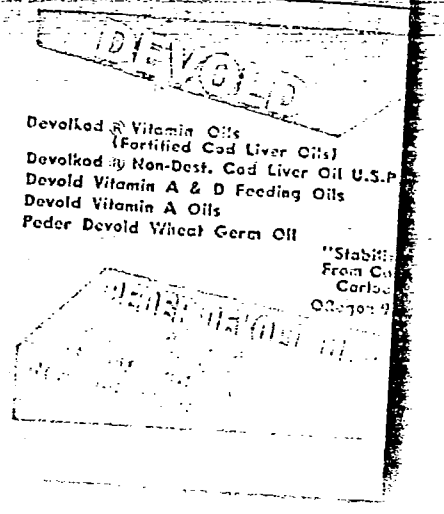
...ation steps and that it was con-
...ted in the residue along with
...non-volatile unsaponifiable sub-
...s. Closer study of the various
...of fatty acid production soon
...ed that the toxic factor was
...ic and was present to some
... in the first distillates which
...used for the production of the
...grades of fatty acids, and those
...ded for food purposes. This dis-
...ery was made independently and
...rted by Ames, et al., who had
...d several samples of oleic acid
... monoglyceride made from such
an oleic acid to be contaminated.

...n this happened just after the
...ssage of the Food Additive
Amendment. The fatty acid manufac-
turing firms were very cooperative
in providing us with information and
samples. However, they sincerely be-
lieved that they were not part of the
food business, that the bulk of their
production went for non-food indus-
trial purposes. In our visits and talks
with their technical people we ad-
vised them to study the applicatio
of the new legislation to their in-
dustry. They realized that a substan-
tial proportion of their highest grad
of materials did enter food channel
when their customers started asking
for guarantees that the fatty acid
met the requirements of the Federa
Food and Drug Law. At that point
the technical committee of the Fatt
Acid Producers Council became ac
tively engaged in a study of thi
problem, and we have enjoyed the
whole-hearted cooperation of the fa
ty acid industry. While they mad
studies to determine what part o
their processes were responsible fo
the production of the toxic materia
studies were continuing on the iso
lation and chemical characterizatio
of the active substance. Three years
ago at the AOAC meeting a sympo
sium on chick edema was held a
which reports from the Purina lab
oratories, the Quaker Oats labora
tory, the Procter & Gamble researc
laboratories and the Food and Dru
laboratories described the progres
made up to that time. The Merc
group had recently entered the pro
lem, and, although they did not re
port, they also had traveled a simila
road.

Every step and experiment had
be followed by the chick bioassa
However, during the first year all th
groups had made steady progress a
about the same rate. First it was
demonstrated that the "chick edema
toxicity was entirely in the unsapon
fiable portion of the fat. Then, in ou
work, the unsaponifiable was sepa
rated by chromatography on alumin
into three fractions of increasing po
larity by elution with petroleum eth
alone, then with a mixture of eth
and petroleum ether and finally wi
100% ethyl ether. The first, or hy
carbon, fraction contained 83% of th
unsaponifiable, and its ultra-viol
absorption spectrum indicated th
presence of cholestadiene. The seco
fraction was characterized

...ected the presence of dipalmit
The third fraction consisted of ste
and oxidized materials. Simultane
bioassay of these three fractions
synthetically prepared samples of
lestadiene and dipalmitone sho
that only fraction 2, but not the
palmitone, was toxic.

Several approaches were tried



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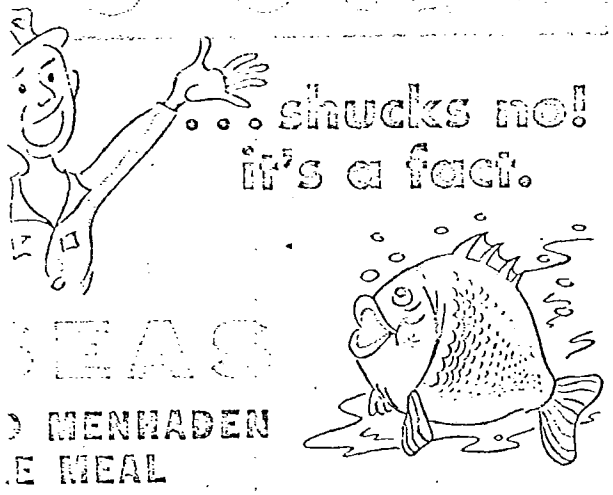

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multaneously to purify fraction 2. I will not take the time to describe our experiments with a 500 tube countercurrent distribution between iso-octane and methanol, or the separation of carbonyls with Girard-T reagent or the consecutive rechromatography on alumina and silane treated Celite. Suffice it to say that as our fractions became more potent we relied more and more on ultra-violet spectrophotometry as an indication of concentration and purity. Two distinct types of fractions were obtained, one with the ultra-violet spectra characteristic of naphthalene compounds and the other of phenanthrene compounds. Both fractions contained the toxic factor. However, the sum of their toxicities was not comparable to that of the starting material in the final purification steps despite the fact that practically all of it was accounted for by weight in the eluates. Since only the fractions that had a significant weight had been tested biologically, the insignificant, hardly visible residue of less than 0.5 mg. in the practically "empty" beaker that represented the cut between the two major fractions was rinsed into a chick diet and to our surprise was very active at approximately 0.1 ppm., representing the most potent material we had obtained. At this stage we had practically exhausted our raw material, and we had to start from the beginning once again.

During the course of these studies we had learned that Drs. Portman and Andrus in the department of nutrition at the Harvard School of Public Health had lost a number of monkeys in a nutritional study. They had used a synthetic triolein as the source of fat and had to abandon the experiment. At a Gordon Research Conference I had the opportunity to discuss this experience with Dr. Portman. Fortunately about 40 lb. of the triolein was still available. It proved to be toxic in the chick edema assay. The triolein was of excellent quality, containing only 0.9% of unsaponifiable material. This unsaponifiable proved to be the richest source of the toxic material we had ever examined.

The toxic fraction was separated from the triglyceride by molecular distillation. The distillate from 17.5 Kg. of triolein was saponified, and the unsaponifiable was chromatographed on alumina to remove the cholestadiene fraction. The naphthalene and phenanthrene-containing cuts were collected, yielding 670 mg. of material. This fraction was rechromatographed on more retentive alumina with 5% ether in petroleum ether, and 240 mg. of naphthalene-phenanthrene material was separated. This was rechromatographed in the same system and three distinct types of U.V. spectra began to emerge in the fractions: Naphthalene (235 mμ), phenanthrene (260 mμ) and a new peak at 245 mμ. These materials were combined and chromatographed on a silane treated Celite-iso-octane column, with 80% alcohol saturated with iso-octane as the mobile phase. The cuts with maximum absorbence at 245 mμ were further purified by two more chromatographic treatments on alumina at a very high sample:adsorbent ratio (1:2,000 and 1:25,000). The final fraction was completely free of absorption peaks near 235 and 260 mμ. This fraction was evaporated to dryness, dissolved in a small volume of boiling iso-octane and stored overnight in the refrigerator. White crystals were obtained by weighing 2.6 mg. and represented a concentration over the original triolein of three million-fold. This material at 1 ppm. in the diet killed chicks in 12 days and produced typical hydropericardium when fed .05 ppm. in a 21-day test.

During the last stages of this work the Merck group [Harmon, et al. JACS 82, 2078 (1960)], announced the isolation of a crystalline chick edema factor from a toxic fat. On July 1, 1960, the Merck group informed us that they had found 47% chlorine in their crystalline material. The presence of chlorine in our material was quickly confirmed by the use of the Dohrmann microcoulometric gas chromatograph.

In this test, a few micrograms of sample is injected into a gas chromatograph and the components as they emerge are pyrolyzed at 800° under oxidizing conditions. The halogen acid formed from halogenated materials is titrated automatically by a microcoulometer.

Of interest also is the isolation of a crystalline material that had the ultra-violet absorption spectrum identical with that of the toxic material but shifted 3 mμ so that the main peak was at 248 mμ instead of 245 mμ. It behaved like the toxic substance in the microcoulometric gas chromatograph, showing a similar retention time and halogen content. However, it was completely inactive in the chick edema test when fed at 1 ppm. in the diet.

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CHICK EDEMA

(Continued from page 24)

toxic substance is a metabolite of a chlorinated insecticide we have fed large doses of chlordane, methoxychlor, heptachlor, aldrin and dieldrin to rats for a month and are feeding the unsaponifiable extract of the rat carcass in the chick test. The results to date show that chlordane and methoxychlor produce no response.

Industrial Experiments

Similar experiments have been carried out and are still in progress by at least two industrial laboratories under conditions of fatty acid production. The results so far have been largely negative or at best only suggestive but not clear cut. Dr. Artman has reported that chlorination of naphthalene and phenanthrene by substitution reactions has produced CEF active products. The chlorinated phenanthrene is particularly promising, since preliminary fractionation experiments indicated the possibility that a highly toxic compound was produced.

Dr. Boyd O'Dell and colleagues at the University of Missouri observed hydropericardium and other symptoms of chick edema disease in chicks housed in freshly painted cages. They traced the responsible agent down to one of the paint ingredients, a chlorinated biphenyl sold under the name Arochlor. We have tested several Arochlors of different chlorine content. Some were not toxic; others produce the disease but only at relatively high feeding levels, e.g., 20% ppm. It may be that an impurity in these compounds is the toxic agent or that they are innately toxic at the high levels fed. Of interest is the use of one of these Arochlors in some insecticide formulations. At the present time there has been no real evidence developed to implicate any product or compound. However, the circumstantial evidence is stimulating considerable speculation and activity.

The feed industry has managed by careful control of ingredients to avoid a recurrence of the 1957 epidemic. The color test developed by Brew, et al., at Purina has been very useful in screening out toxic fats. This test is not specific but is useful since the presence of large amounts of steroidal compounds that give this test usually indicates still residues that may be toxic. It is useless, however, for fatty acids and other products such as triolein, monoglycerides, etc., that give no response in this test but which sometimes are quite toxic.

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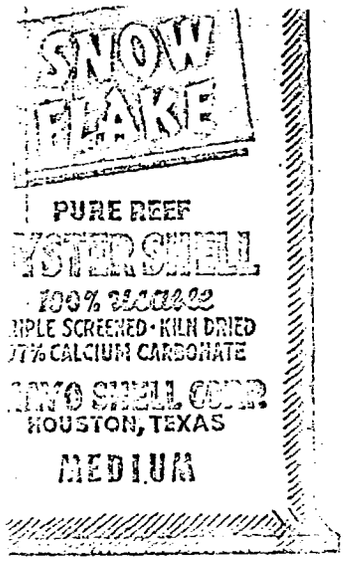

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substance was a major breakthrou
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in terms of a naturally occurring n
terial that had changed chemica
under the conditions of industr
fatty acid production. The possibil
of contamination with one of ma
familiar chlorinated hydrocarb
was obvious. Samples of tetrachlo
naphthalene and hexachlorona
thalene, kindly provided by En
and Bell of Virginia Polytechnic
stitute, who had demonstrated th
these compounds produced hyp
keratosis in cattle, were without
fect in the chick edema test. We h
tested a long list of chlorinated c
pounds including aldrin, dieldrin,
dane, DDT, DDE, BHC, chlord
toxaphene, methoxychlor, and a se
of Halowaxes, without any definit
positive indication. Furtherm
these have all been heated with c
acid at 250° C. for long periods
then fed to chicks, with negative
sults. Also, on the theory that ta
may sometimes be bleached with
tive chlorine materials which
chlorinate a sterol nucleus, we l
chlorinated cholesterol, squalene,
tone and equilenin with negative
sults. To test the theory that

(Turn to CHICK EDEMA, page 71

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Last year, at this time, on outbr of the chick edema disease occur in Georgia. Although considera chlordane residues were found in feed, we do not believe they were sponsible for the symptoms obser Furthermore, the best informat we have indicates that only rende fat was used in this feed and no pr uct of the fatty acid industry v involved. With each new developm the scope of the problem increa First we were concerned only w still residues, then also with fa acid distillates and their derivati For a long time it was felt that o the fat derived from animals was volved. Recent evidence from sour in the fatty acid industry and own studies indicates that some ve table fat sources may yield fa acids contaminated with CEF.

Still another development has curred to further complicate the ture. Early this year in the course our regulatory activities, we exa ined a sample of oleic acid. All test chicks died by the end of second week, with symptoms of verely stunted growth, ascites, ja dice and pathology of the liver a other organs, but with no hydrop cardium. This sample had been tes by the manufacturer last year bef the adoption of the present AO. test procedure. The testing laborat had used the procedure we oursel

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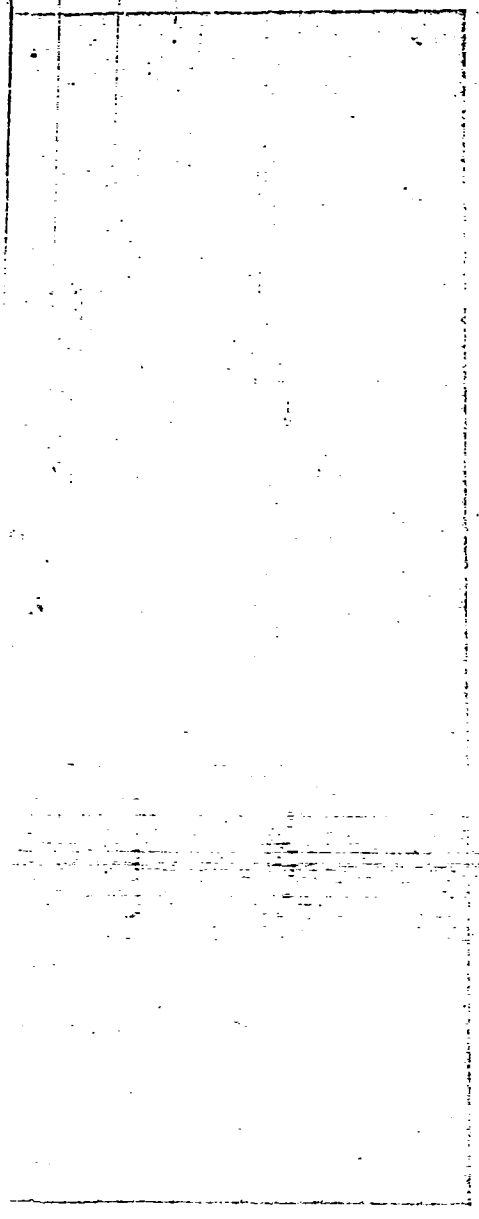

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had used in our earlier work, and had found the sample negative for chick edema. Repeat of the test in both laboratories by both procedures confirmed both findings. The sample is free of chick edema disease factor when tested on a diet of natural ingredients, and the chicks survive in apparent good health. On the casein-sucrose diet of the AOAC chick edema test, the chicks fail to grow and die early with the described symptoms but no hydropericardium. Furthermore, the dose response curve for this effect is very steep, since the ratio of the dose that gives a maximum effect to the dose that produces a minimum effect is less than two, as compared to a ratio of four to five for chick edema factor. Evidence from preliminary fractionation studies also indicates that this is an entirely different substance. There is no other information as to its characteristics at this time. There is evidence that this toxic contamination has occurred in different places from time to time and in a variety of fatty acid samples and may occasionally occur together with CEF. Here again we must anticipate that this material may occur elsewhere independently of the fatty acid industry.

For whatever comfort we may derive, it should be noted that chick edema disease has been observed in England. In a letter to the editor of the Veterinary Record of June 10, 1961, C. C. Wannop of the Houghton Poultry Research Station draws attention to a condition apparently identical with that reported by Sanger, et al., and Schnittle, et al., in 1958, that has appeared in several broiler flocks. In a personal note dated Sept. 29, he says that the condition has disappeared for the time in his country.

At the present time fatty acids can be used in the manufacture of foods or food ingredients only if they are free from CEF. This requirement made necessary the development of a bioassay which has been accomplished by collaborative work and is now being adopted as official by the AOAC.

I have tried to review the history of this troublesome problem, what little is known of its toxicology and its physiological aspects, the speculations as to the origin of the contamination, and attempts to track down its sources. I have sketched quickly our own attempts at isolation and identification of the toxic factor and have alluded to the most recent developments along this line that were reported at the AOAC Section of Fats and Oils by Dr. Artman.

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