cte
aff Inc.
Chemists
LD, MO.
-2851

TALK
FITS

UPPLIER—
eally do
NESS
ASH!
f feedmen
ve
n do it
Daffin!
n labor at
more & more
ders pay
or work
farm
y used to
nselves!
lcome the
ead service
obile Mills.
MONS 029540

Progress in the Chick Edema Problem

By Dr. Leo Friedman
Food and Drug Administration

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.

Symptoms Different

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

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 as to the effect of "chick edema 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

reduction in growth without hydr pericardium or ascites."

In chicks, "Microscopic examin tion of the tissues of the test anim revealed lymphocytic foci in the e cardium and myocardium. Edem tous fluid separated the myocardi fibers. Edema of the lungs was a fr quent observation in the experime tal birds.

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

"'Toxic fat' produces a reduct in growth rate of experimental a mals. This reduction depends on age of the animal and the level 'toxic fat' added to the diet. Hy pericardium and ascites are a quent lesion in the animals recei from 1.0 to 5.0% 'toxic fat.' W this level was reduced to 0.25% the diet, reduced testicular deve ment was a more sensitive crit than hydropericardium, ascites weight gain for evaluating chr toxicity.

"T mechanism by which 't fat' induces hydropericardium ascites appears to be associated degeneration and edema of the r cardial fibers. These data would to eliminate the kidneys, liver endocrines as the primary cau edema. The early development of dropericardium, increased ven pressure, enlarged hearts, mitoc drial changes in the myocardium generalized edema suggest that myocardium may be directly in ed; however, altered capillary p ability has not been excluded. It lieved that cardiac decompe and increased capillary perma act together in producing the sive extravascular fluid collec the demise of the animal."

The wide range of suscep within and among species variety of toxic effects that hav ready been noted would make year logical that some primary of structure and function such mitochondrion, may be the tar the toxic factor and that the ob differences may be explained b tors such as absorption, specific ing, transport, detoxication, etc determine the local concentrat any substance in a specific si

Another possible mechanism had suggested itself quite e that the toxic factor interfere the normal regulation of elect and water balance. Selye and back in 1943, described the

ALK
ITS
UPPLIER—
eally do
NESS
ASH!
of feedmen
ove
n do it
Daffin!
labor at
more & more
ders pay
or work
farm
y used to
nselves!
come the
ed service
lobile Mills.
ice your credit
gh inventory,
-over.
r Share
business
RE • PHONE
CTURING
PRINCE ST.
TER, FA.

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.

Symptoms Different

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 group 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 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 as to the effect of "chick edema 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

in growth rate of ... mals. This reduction ... are of the animal and ... 'toxic fat' added to the ... pericardium and ascites ... quent lesion in the animals ... from 1.0 to 5.0% 'toxic fat' ... this level was reduced to ... the diet, reduced testicular ... ment was a more sensitive cri... than hydropericardium, ascites, ... weight gain for evaluating ... 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 causes of edema. The early development of hydropericardium, increased venous pressure, enlarged hearts, mitochondrial changes in the myocardium, generalized edema suggest that the myocardium may be directly involved; however, altered capillary permeability has not been excluded. It is believed that cardiac decompensation and increased capillary permeability act together in producing the massive extravascular fluid collection and the demise of the animal."

The wide range of susceptibility within and among species and variety of toxic effects that have already been noted would make it a year logical that some primary site of structure and function such as the mitochondrion, may be the target of the toxic factor and that the observed differences may be explained by factors such as absorption, specific binding, transport, detoxication, etc., which determine the local concentration of any substance in a specific site.

Another possible mechanism that had suggested itself quite early is that the toxic factor interferes with the normal regulation of electrolyte and water balance. Selye and Stone back in 1943, described the production of edema symptoms in chicks by certain steroids and the accentuation of these symptoms by increased salt intake of the chicks. Alcock has shown that it is possible to produce hydropericardium in chicks by increasing the NaCl in the ration and to prevent its occurrence even with CEF by eliminating NaCl from the diet. Work in our own and other laboratories has illustrated beautifully the interaction of nutritional factors on the susceptibility of the chick to a toxic agent. For example, the current AOAC bioassay diet for "edema factor" is probably many times more sensitive than the assay diet we used originally, although both have approximately the same protein content.

A point to remember is that hydropericardium as a symptom of toxicity in chicks is not new. In addition to NaCl and certain steroid hormones, chapter 40 in Biester and Schwarte on Poisons and Toxins indicates that (1) Zinc phosphide, used as a

(Continued on page 22)

identical 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 noticed 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.

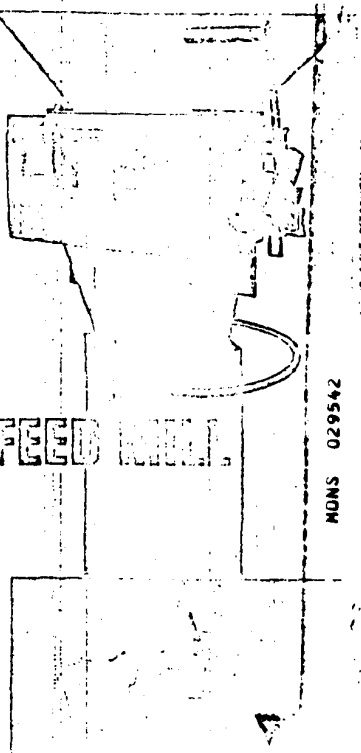
It is well known now that chicks can efficiently utilize large amounts of fat in properly balanced rations. The use of fat as a standard ingredient of poultry feeds grew as the price of fat calories dropped and became competitive with calories derived from corn. The chick edema disease epidemic of 1957 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 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

7 Ways Richardson G-17 Semi-Automatic Baggers

SAVE MONEY IN ANY FEED MILL



1. FIT ANY SIZE MILL—as the main bagger for the small mill or the small-batch bagger for the larger mill.
2. HANDLE MOST FEEDS—grains, pellets, mashes.
3. HANDLE ALL SIZES AND TYPES OF BAGS—open-mouth textile or multi-wall paper—10 to 140 lb. capacities.
4. LOW COST—little more than an all-manual unit.
5. AUTOMATIC CUT-OFF—no time lost trimming . . . much faster than all-manual baggers.
6. CONSISTENT ACCURACY—eliminates human error . . . keeps profits out of the bag and in your pocket.
7. COMPLETE LINE OF ACCESSORIES.

Bagholders—camgrip (shown); also manual and air-operated.
Spouts—quick-change square or oval in a range of sizes.
Feeder—increases speed and accuracy—permits handling flakes,

MONS 029542

various degrees
he accumulation
n the pericardial
-abdominal cav-

yl thiouracil, the
ause in poisoned
ning edema and
fluid in the peri-

methanesulfonate,
ined very effec-
ainess in chicks
ericardial sac with
luid, in addition
ological changes
ngs.

he primary le-
at cases were in
e quantities of
the pericardial

ckie" and several
s produce seeds
n in chicks the
-toxic hydroperi-

ease other char-

acteristic 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 acid 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

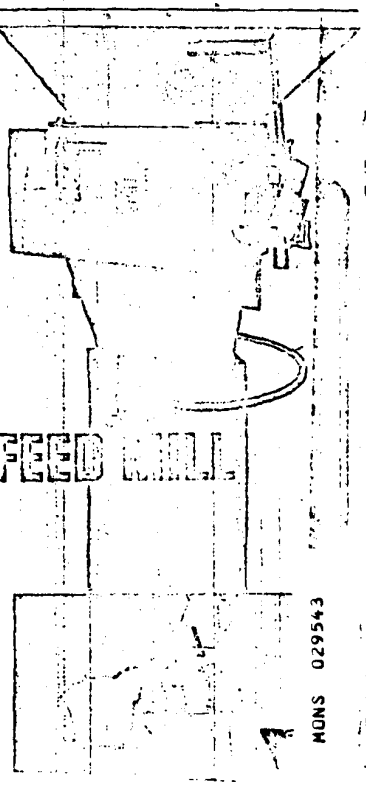
...ation steps and that it was ...tained in the residue along ...ther non-volatile unsaponifiable ... Closer study of the ... of fatty acid production ...ealed that the toxic factor ...ifiable and was present to ...xtent in the first distillates ... were used for the production of ...est grades of fatty acids, and ... to feed for food purposes. The ...overy was made independently ...reported by Ames, et al., who ...found several samples of oleic ... monoglyceride made from ...ic acid to be contaminated ...

All this happened just after ...sage of the Food Addi... Amendment. The fatty acid manufacturing firms were very cooperative in providing us with information ... samples. However, they sincerely believed that they were not part of the food business, that the bulk of ... production went for non-food industrial purposes. In our visits and ... with their technical people we ...lped them to study the applica... of the new legislation to their ...ustry. They realized that a substantial proportion of their highest ... of materials did enter food channels when their customers started asking for guarantees that the fatty ... met the requirements of the Food ... Food and Drug Law. At that ... the Technical committee of the ... Acid. Producers Council became ...tively engaged in a study of ... problem, and we have enjoyed ... whole-hearted cooperation of the ...ty acid industry. While they ... studies to determine what part ... their processes were responsible ... the production of the toxic mate... studies were continuing on the ...lation and chemical characteriza... of the active substance. Three ... ago at the AOAC meeting a sym... sium on chick edema was held ... which reports from the Purina ...oratories, the Quaker Oats Lab...tory, the Procter & Gamble re... laboratories and the Food and ... laboratories described the ... made up to that time. The ... group had recently entered the ...lem, and, although they did not ... port, they also had traveled a si... road.

Every step and experiment ... be followed by the chicks bio... However, during the first year ... groups had made steady progress ... about the same rate. First it ... demonstrated that the "chick e... toxicity was entirely in the unsa... fiable portion of the fat. Then, ... work, the unsaponifiable was ... rated by chromatography on a ... into three fractions of increasing ... larity by elution with petroleum ... alone, then with a mixture of ... and petroleum ether and finally ... 100% ethyl ether. The first, or ... carbon, fraction contained F... unsaponifiable, and its ... ...be separated on special ... ...ne ... of cholestadiene ... ethanol about ...

ays
Richardson G-17
mi-Automatic
ggers
MONEY IN ANY FEED MILL

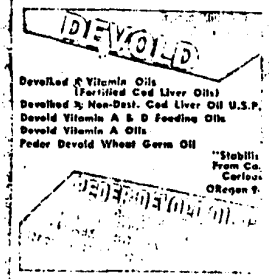
MILL—as the main bagger for the small mill or the ...ave for the larger mill.
FEEDS—grains, pellets, meshes.
IZES AND TYPES OF BAGS—open-mouth textile or ...r—10 to 140 lb. capacities.
...le more than an all-manual unit.
...-OFF—no time lost trimming... much faster than ...ers.
CCURACY—eliminates human error... keeps profits ...el in your pocket.
E OF ACCESSORIES
...-clamp-grip (shown); also manual and air-operated.
...k-change square or oval in a range of sizes.
...reases ...ined and accuracy—permits handling flakes,



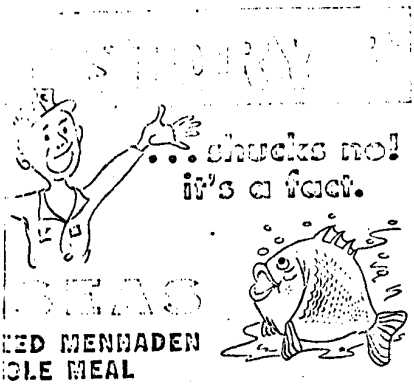
MONS 029543

[illegible] 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



MONS 029544



...ED MENHADEN
...OLE MEAL
...of Condensed Fish Solubles)

in fish growth factors
in availability of amino acids
in metabolizable energy values

... the quality of fishmeal or solubles proc-
Haynie. First-in-the-field research has devel-
Haynie a protein which can be *guaranteed* for

no finer product—and no better protein value
market.

Haynie

multaneously to purify fraction 2. I will not take the time to describe our experiments with a 500 tube counter-current 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 [illegible] distillation. The distillate from [illegible] kg. of [illegible] was saponified [illegible] the unsaponifiable was chromatographed on alumina to remove [illegible] cholestadiene fraction. The naphthalene and phenanthrene-containing cuts were collected, yielding [illegible] of material. This fraction was [illegible] chromatographed on more [illegible] alumina with 5% ether in petrol [illegible] 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 [illegible] 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 in two more chromatographic treatments on alumina at a very high sample:adsorbent ratio (1:2,000 and 25,000). The final fraction was completely free of absorption peaks [illegible] 235 and 260 mμ. This fraction was evaporated to dryness, dissolved [illegible] a small volume of boiling iso-octane and stored overnight in the refrigerator. White crystals were [illegible] weighing 2.6 mg. and [illegible] concentration over the [illegible] lein of [illegible] rial at [illegible] chicks in 12 days and produced [illegible] cal hydropericardium when [illegible] .05 ppm. in a 21-day test.

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

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

Of interest also is the isolation [illegible] a crystalline material that had [illegible] ultra-violet absorption spectrum [illegible] tical with that of the toxic mate[illegible] but shifted 3 mμ so that the m[illegible] peak was at 248 mμ instead of [illegible] mμ. It behaved like the toxic [illegible] stance in the microcoulometric chromatograph, showing a similar [illegible] tention time and halogen cont[illegible] However, it was completely inac[illegible] in the chick edema test when [illegible] 1 ppm. in the diet.

The finding of chlorine in the [illegible] substance was a major breakth[illegible] It was no longer necessary [illegible] in terms of a naturally [illegible] terial that had [illegible] under the condi[illegible]

50 LBS. NET
...YO'S
NEW-IMPROVED

You Better Believe It...
When You SELL
"SNOW FLAKE"

MONS 029545

in fish growth factors
in availability of amino acids
in metabolizable energy values

...the quality of fishmeal or solubles proc-
Haynie. First-in-the-field research has devel-
Haynie a protein which can be *guaranteed* for

no finer product—and no better protein value
market.

Haynie

Baltimore 30, Maryland

jor 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

235 and 260 mμ. This fra... evaporated to dryness, dis... a small volume of boiling ... and stored overnight in the ... tor. White crystals were ... weighing 2.6 mg. and ... concentration over the ... lein of three million-fold ... rial at 1 ppm. in the ... chicks in 12 days and produced ... cal hydropericardium when f... .05 ppm. in a 21-day test.

During the last stages of this the Merck group [Harmon, ... JACS 82, 2078 (1960)], announce... isolation of a crystalline chick ... factor from a toxic fat. On Ju... 1960, the Merck group inform... that they had found 47% chlor... their crystalline material. The ... ence of chlorine in our materia... quickly confirmed by the use ... Dohrmann microcoulometric chromatograph.

In this test, a few microgra... sample is injected into a gas ... matograph and the componen... they emerge are pyrolyzed at ... under oxidizing conditions. The ... gen acid formed from haloge... materials is titrated automatica... a microcoulometer.

Of interest also is the isolati... a crystalline material that ha... ultra-violet absorption spectrum ... tical with that of the toxic mat... but shifted 3 mμ so that the ... peak was at 248 mμ instead of ... mμ. It behaved like the toxic ... stance in the microcoulometric chromatograph, showing a simil... tention time and halogen con... However, it was completely ina... in the chick edema test when f... 1 ppm. in the diet.

The finding of chlorine in the ... substance was a major breakthr... It was no longer necessary to ... in terms of a naturally occurring ... terial that had changed chemi... under the conditions of indu... fatty acid production. The possi... of contamination with one of ... familiar chlorinated hydroc... was obvious. Samples of tetrach... naphthalene and hexachloro... thalene, kindly provided by ... and Bell of Virginia Polytechni... stitute, who had demonstrated ... these compounds produced h... keratosis in cattle, were withou... fect in the chick edema test. We ... tested a long list of chlorinated ... pounds including aldrin, dieldrin ... dane, DDT, DDE, BHC, chlor... toxaphene, methoxychlor, and a ... of Halowaxes, without any ... positive indication. Furth... these have all been heated with ... acid at 250° C. for long period... then fed to chicks, with ne... sults. Also, on the theory th... may sometimes be bleached ... tive chlorine materials which ... chlorinate a sterol nucleus, we ... chlorinated cholesterol, ... tone and equilenin with ne... sults. To test the theory ...

(Turn to CHICK EDEMA, ...

50 LBS. NET
MAYO'S
NEW-IMPROVED
SNOW FLAKE
PURE REEF
100% ...
IPLE SCREENED-KILN DRIED
1% CALCIUM CARBONATE
MAYO SHELL CORP.
HOUSTON, TEXAS
MEDIUM

You Better Believe It...

When You SELL
"SNOW FLAKE"
You SELL the BEST

This We Guarantee

Write—Wire—'Phone
for PRICES and SAMPLES

MONS 029546

MAYO SHELL CORPORATION
P. O. BOX 704 HOUSTON 1, TEXAS TEL: OR 2-9441

at steam. In
ingredients are
ely low mois-
ment can be
ter drip-feed
at needed. A
scoop feeding.

soline power
a companion
o the electric
introduced by
which is used
ch and farm.
facturer.

h take-off, is
l or battery
and is said to
et production
formula grain
xtures.

sizes that the
and requires
It will, how-
from grain
whole. Eight
ng from three
ve eighths in.
e will occupy

a floor space of 3 by 3 ft. and weighs less than 175 lb.

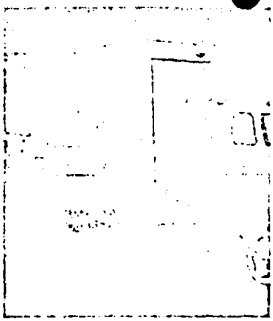
For additional information check No. 7900 on the coupon and mail.

No. 7903—Concentrate Carrying Mobile Mills

Daffin mobile feed mills are now available in models equipped to carry up to 2 tons of feed concentrates, minerals, etc.

Mounted on heavy-duty, "cab-forward" chassis with 153 in. wheelbases and heavy-duty springs, the concentrate-carrying Feedmobiles are available in three styles of factory installed carriers.

These are a platform-type, equipped with non-slip deck-plating in the space between the truck cab and the diesel power unit; the stake-type carrier rigged with heavy-duty stakes and chain railings to hold bagged concentrates during transit, and a compartment-type carrier, which is a completely enclosed aluminum van compartment with two opposite-side access doors.



The concentrate carriers are being installed only on Feedmobiles delivered at the factory for which the special trucks are specified.

For additional information check No. 7903 on the coupon and mail.

CHICK EDEMA

(Continued from page 21)

toxic substance is a metabolite o chlorinated insecticide we have large doses of chlordane, metho chlor, heptachlor, aldrin and diel to rats for a month and are feed the unsaponifiable extract of the carcass in the chick test. The res to date show that chlordane and m thoxychlor produce no response.

Industrial Experiments

Similar experiments have been c ried out and are still in progress at least two industrial laborator under conditions of fatty acid prod tion. The results so far have be largely negative or at best only s gestive but not clear cut. Dr. Artir has reported that chlorination naphthalene and phenanthrene substitution reactions has produ CEF active products. The chlorinat phenanthrene is particularly prom ing, since preliminary fractionat experiments indicated the possibil that a highly toxic compound w produced.

Dr. Boyd O'Dell and colleagues the University of Missouri observ hydro[illegible]icardium and other sym toms of chick edema disease in chic housed in freshly painted cages. Th traced the responsible agent down one of the paint ingredients, a chlo nated biphenyl sold under the na Arochlor. We have tested seve Arochlors of different chlorine co tent. Some were not toxic; others pr duce the disease but only at rel tively high feeding levels, e.g., 2 ppm. It may be that an impurity these compounds is the toxic ager or that they are innately toxic at t high levels fed. Of interest is the u of one of these Arochlors in some i secticide formulations. At the prese time there has been no real evider developed to implicate any produ or compound. However, the circu stantial evidence is stimulating co siderable speculation and activity.

The feed industry has managed careful control of ingredients to avo a recurrence of the 1957 epidemi The color test developed by Brew, al., at Purina has been very usef in screening out toxic fats. This te is not specific but is useful since th presence of large amounts of steroid compounds that give this test usual indicates still residues that may toxic. It is useless, however, for fat acids and other products such as tr lein, monogylcerides, etc. that g no response in this test, but [illegible] sometimes are quite toxic.

Georgia Outbre...

Last year, at this time, an outbre of the chick edema disease occur in Georgia. Although consider chlordane residues were found in feed, we do not believe they were sponsible for the symptoms ob Furthermore, the best inf we have indicates that only [illegible]

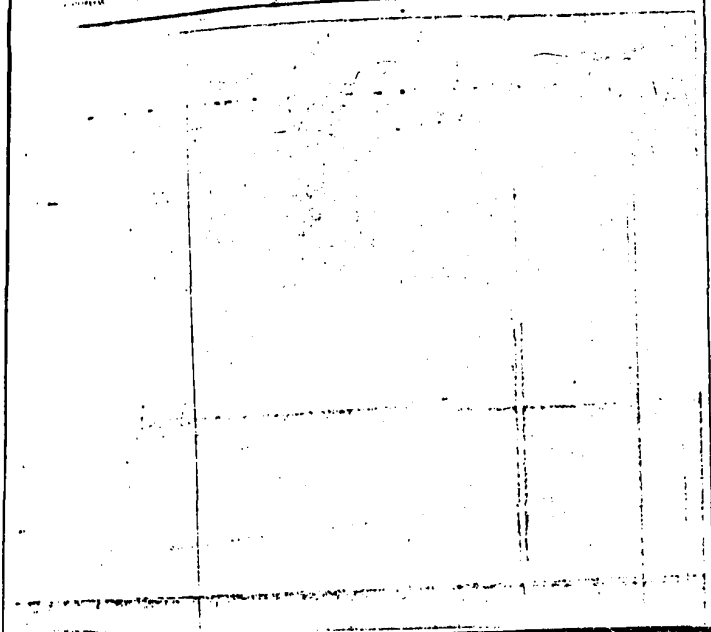
MONS 029547

[illegible] may be decided on this spot!

[illegible] may be the site [illegible]tion of your new [illegible] decisions. [illegible]

at precisely this point. Our experience in designing and manufacturing equipment, plus our background of complete feed mill engineering assures you of maximum operating efficiency throughout the many years ahead.

... compartment-type ... is a completely enclosed alumi- | No. 7903 on the coupon and ...



experiments indicated the ... that a highly toxic compound produced.

Dr. Boyd O'Dell and colleagues ... the University of Missouri ... hydro-pericardium and other ... toms of chick edema disease in ... housed in freshly painted cages. ... 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 steroid 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 ... lein, monogylcerides, etc. ... no response in this test, but ... sometimes are quite toxic.

... future may be decided on this spot!

... like it . . . may be the site ... construction of your new ...

... two important decisions. ... to build a mill. Second ... you're going to put it.

... the most important task of ... what kind of mill you ... engineered, constructed, and ... can get the best value for ... entire business future may ... you make now.

... possible profit from *any* feed ... the location . . . you must ... efficient facility. That's ... call in Myers-Sherman at precisely this point. Our experience in designing and manufacturing equipment, plus our background of complete feed mill engineering assures you of maximum operating efficiency throughout the many years ahead.

Working with you or your board, we'll submit flow charts, cost estimates, engineering plans and, if you wish, handle the contracting at no extra cost to you. Your new mill will be designed and built to produce the greatest possible volume and profit at the lowest possible operating cost.

You have to have a PLAN before you can have a PLANT . . . and that's where we can save you both time and money. Send today for our free booklet that tells you all about the Myers-Sherman concept of ECONOMATION-ENGINEERED feed mill planning and construction.

MONS 029548

MYERS-SHERMAN COMPANY

... STREET • ... TOR, ILLINOIS • PHONE 2-2176

Georgia Outbreak

Last year, at this time, on outbreak of the chick edema disease occurred in Georgia. Although considerable chlordane residues were found in the feed, we do not believe they were responsible for the symptoms observed. Furthermore, the best information we have indicates that only rendered fat was used in this feed and no product of the fatty acid industry was involved. With each new development the scope of the problem increased. First we were concerned only with still residues, then also with fatty acid distillates and their derivatives. For a long time it was felt that only the fat derived from animals was involved. Recent evidence from sources in the fatty acid industry and our own studies indicates that some vegetable fat sources may yield fatty acids contaminated with CEF.

Still another development has occurred to further complicate the picture. Early this year in the course of our regulatory activities, we examined a sample of oleic acid. All test chicks died by the end of the second week, with symptoms of severely stunted growth, ascites, jaundice and pathology of the liver and other organs, but with no hydropericardium. This sample had been ... by the manufacturer last year ... the adoption of the present ... test procedure. The testing laboratory had used the procedure we ...

HAMMERMILLS
AND FEED MILL EQUIPMENT

HEAVY DUTY
LOW PIT
DRAG FEEDER



Particularly desirable where dump pit is shallow or water is a problem.

MACHINE WORKS, INC.

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 Schmittle, 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.

BIBLIOGRAPHY

Chick "Edema" Disease (Hydropericardium):

1. Sanger, V. L., et al.—J. Am. Vet. Med. Assn., 133, 172 (1958), "Alimentary toxemia in chickens."
2. Edgar, S. A., et al.—Poultry Sci., 3[illegible], 1200 (1958), "Effect of a toxic substance in fat on poultry."
3. Naber, E. C., et al.—Poultry Sci., 3[illegible], 1229 (1958), "Effect of certain toxic fats and their derivatives on growth, reproductive performance, embryonic development and health of chickens."
4. Schmittle, S. C., et al.—Georgia Poultry Disease Research Center, Athens, [illegible] (1958), "Progress report on toxic fat disorder of chickens."
5. Schmittle, S. C., et al.—J. Am. Vet. Med. Assn., 132, 216 (1958), "A disorder of chickens probably due to a toxic feed preliminary report."
6. Brew, W., et al.—J. Assoc. Off. Agric. Chem., 42, 120 (1959), "Characterization of a type of unidentified compound producing edema in chicks."
7. Friedman, L., et al.—J. Assoc. Off. Agric. Chem., 42, 129 (1959), "Studies on chicken edema disease factor."
8. Wootton, J. C., et al.—J. Assoc. Off. Agric. Chem., 42, 141 (1959), "Some chemical characteristics of the chicken edema disease factor."
9. Potter, G. C., et al.—J. Am. Oil Chem. Soc., 36, 214 (1959), "Current status of toxic principle causing the chick edema syndrome."
10. Anon.—Feedstuffs, 30, 21, 1 (1958), "American Feed Manufacturers Assn. special report on edema in chickens."
11. Wilder, O. H. M., and Dugan, L. R.—Amer. Meat Institute Foundation Special Report (1958), "Progress in certain animal fats relating to a poultry disease."
12. Machlin, L. J., et al.—Poultry Science, 38, 579 (1959), "Relationship of oxidative degradation to toxicity in certain [illegible]."
13. Dunahoo, W. S., et al.—Poultry Science, 38, 663 (1959), "Studies on toxicity in rations of laying hens and pullets."
14. Edgar, S. A., et al.—47th Meeting Poultry Science Assoc., Ithaca, N.Y., August, 1958, "The effect of a toxic [illegible]



He looks right through

Crest Trace-Mineral Pre-Mixes . . .

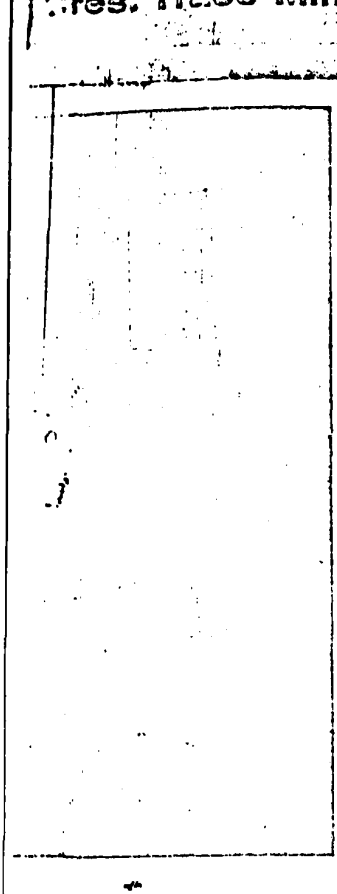
The Lime Crest spectrographer carefully "reads" spectrogram after spectrogram just to be sure that *Lime Crest* Trace-Mineral Pre-Mixes meet the guarantees that have been made for them. Nothing is left to chance . . . each pre-mix *must* be right. That's why you can depend on the trace-mineral pre-mixes you buy from Lime Crest. But Lime Crest has much more than painstaking control to offer. Almost 15 years ago the Lime Crest Research Laboratory was established — the only research Laboratory in the industry operated primarily to study the role of trace minerals in animal nutrition and to develop better trace-mineral pre-mixes. From the Lime Crest Research Laboratory have come many important developments: new and better ingredients of trace-mineral pre-mixes — ingredients which are not only fully compatible with one another but also more compatible with the fat-soluble vitamins

MONS 029549



He looks right through

Crest Trace-Mineral Pre-Mixes.



The Lime Crest spectrographer carefully "reads" spectrogram after spectrogram just to be sure that Lime Crest Trace-Mineral Pre-Mixes meet the guarantees that have been made for them. Nothing is left to chance . . . each pre-mix *must* be right. That's why you can depend on the trace-mineral pre-mixes you buy from Lime Crest. But Lime Crest has much more than painstaking control to offer. Almost 15 years ago the Lime Crest Research Laboratory was established — the only research Laboratory in the industry operated primarily to study the role of trace minerals in animal nutrition and to develop better trace-mineral pre-mixes. From the Lime Crest Research Laboratory have come many important developments: new and better ingredients of trace-mineral pre-mixes — ingredients which are not only fully compatible with one another but also more compatible with the fat-soluble vitamins in your feed. And Lime Crest Trace-Mineral Pre-Mixes remain free-flowing indefinitely; they do not cake or "set". If you are not already using a Lime Crest Trace-Mineral Pre-Mix*, write or call us for more information . . . at least, let us send you our latest folder and price list.

**If you want a special pre-mix, we can satisfy your requirements . . . send us your specifications for a prompt quotation.*

MONS 029550

LIME CREST

TRACE MINERAL PRE-MIXES

Vulcan Corp. of America, Newton, N.J. — World's Largest Producer of Crystalline Calcite Products
Distributor: The Ash Chemical Company, P.O. Box 14, Richelieu, Province of Quebec

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 trace 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. These were reported at the AOAC Section of Fats and Oils by Dr. Arnold.

BIBLIOGRAPHY

Chick "Edema" Disease (Hydropericardium):

1. Bunger, V. L., et al.—J. Am. Vet. Med. Assn., 133, 172 (1958), "Alimentary toxemia in chickens."

2. Edney, R. A., et al.—Poultry Sci., 37, 1200 (1958), "Effect of a toxic substance in fat on poultry."

3. Nelson, R. C., et al.—Poultry Sci., 37, 1329 (1958), "Effect of certain lower fatty acids and their derivatives on growth, reproductive performance, embryonic development and health of chickens."

4. Schmittle, S. C., et al.—Livestock Poultry Disease Research Center, Athens, Ga. (1958), "Progress report on toxic fat disorder of chickens."

5. Schmittle, S. C., et al.—J. Am. Vet. Med. Assn., 132, 210 (1958), "A disorder in chickens probably due to a toxic feed preliminary report."

6. Brow, W., et al.—J. Assoc. Off. Agr. Chem., 42, 129 (1959), "Characterization of a type of unidentified compound producing edema in chicks."

7. Friedman, L., et al.—J. Assoc. Agric. Chem., 42, 328 (1959), "Studies on chicken edema disease factor."

8. Wentink, J. C., et al.—J. Assoc. Agric. Chem., 42, 341 (1959), "Some chemical characteristics of the chicken edema disease factor."

9. Potter, G. C., et al.—J. Am. Oil Chemists' Soc., 36, 214 (1959), "Current status of toxic principle causing the chick edema syndrome."

10. Anon.—Feedstuffs, 30, 21, 1 (1958), "American Feed Manufacturers Assn. to issue special report on edema in chickens."

11. Wilder, O. H. M., and Ingalls, L. B. Amer. Meat Institute Foundation special Report (1958), "Progress in certain unidentified factors relating to a poultry disease."

12. Machlin, L. J., et al.—Poultry Science, 38, 529 (1959), "Relationship of active degradation to toxicity in certain fats."

13. Donahoe, W. S., et al.—Poultry Science, 38, 603 (1959), "Studies on toxic fats in rations of laying hens and pullets."

14. Edney, R. A., et al.—17th Annual Poultry Science Assoc. Meeting, N.Y., August, 1958, "The effect of a toxic substance on fat in poultry."

15. Amos, H. E., et al.—J. Am. Oil Chemists' Soc., 37, No. 4 page 18, April, 1960, "The occurrence of the chick pericardial oedema factor in some oleic acids and products derived therefrom."

16. Harman, R., et al.—J. Am. Oil Chemists' Soc., 37, 2073 (1960), "The isolation and characterization of the chick edema factor."

17. Harman, R. E., Davis, G. K., Orr, H., Brink, N. G., and Riddell, W. A.—J. Agric. Chem. Soc., 37, 2073 (1960).

18. Wertzoff, A., Fitz-Hugh, D., Rumbaugh, W., and Friedman, L.—J. Am. Chem. Soc., 38 (2), 68-82 (1961).

19. Firestone, D., Horwitz, W., Feldstein, L., and Shue, C. M.—J. Am. Oil Chemists' Soc., 38, 418-422 (1961).

20. Douglass, C., and Flick, D. F.—J. Assoc. Offic. Agri. Chemists, 44 (3), 1 (1961).

21. Shue, G. M., and Cutts, L.—Ibid; 59.

22. Flick, D. F., Cutts, L., Wiebe, Douglass, C. D., and Friedman, L.—J. A. in press.

23. Anon., James Dick, Jr.—Dissertation Abstracts, 22 (3), 815 (1961).

24. Wheaton, J. G., Alexander, J. C., Anon, R.—Progress Report on Studies of Chick Edema Factor, presented at the Assoc. Offic. Agri. Chemists' Oct. 1961, Washington, D.C. (in press).

25. McGhee, J. E., Jr., Bird, H. R., Orbell, R. L.—Pre-publication report, University of Missouri, Columbia, Mo.—"A periodation and assay in chicks of chlorinated hydrocarbons."

26. Orr, W. H., Dickinson, A. G., Van Libertine, A.—"Poultry Sci. (1961), "A chick assay for edema producing factor in toxic