The comparative absence of radiation damage and radiation decomposition products in the tritiation of saturated fatty acid esters was quite unexpected in view of the complexity of radiochemical reaction products reported by Wilzbach (5) for hexane and cyclohexane.

It is concluded that saturated fatty esters may be easily labelled with tritium in high specific activity by the Wilzbach procedure of gas exposure and that both the standard procedures for fatty acid purification and the alcohol distillation for removal of exchangeable tritium are effective.

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Current Status of the Toxic Principle Causing the Chick Edema Syndrome

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THE BROILER INDUSTRY was faced with a new malady, which appeared in epidemic proportions during the fall of 1957. Reports of the syndrome had been made earlier in the year, but the number of birds involved was quite small and the condition apparently disappeared spontaneously.

The losses of birds by broiler raisers reached some thousands of birds per day at the peak of the trouble. The birds began to die at three to four weeks of age, and post-mortem examination revealed the pericardial sac surrounding the heart almost always distended with fluid. As the condition progressed, the abdominal cavity quite often was filled with ascitic fluid, which led to the common term "water belly" as a name for the condition (Figure 1).

In the young chick the symptoms of this trouble included gasping, poor weight gain, paleness, and a waddling duck-like gait as the abdomen began to fill with fluid. Sudden deaths occurred in the affected flocks at three to four weeks of age.

The symptoms have not been observed to be as severe in the adult chicken. Laying hens suffered from a drop in egg production and fertility of the eggs. The typical condition of edema has not been produced in turkey poults and ducks when they were fed diets containing the toxic material. Laboratory rats, calves, and pigs did not exhibit the symptoms of edema when kept on diets containing the toxic material. White rats fed a broiler ration which was toxic to chickens did show some depression in growth.

Sanger *et al.* (1) reported that the fluids collected from the abdomen and pericardial cavity of birds suffering from the edema symptoms were sterile. No organism of a significant nature was recovered from cultures of liver, heart, and lung. Intestinal washings and fluid from the heart and abdomen appeared to be nontoxic when injected into white mice. Whole intestinal tracts homogenized and fed to white mice and four-week-old broilers for several days had no toxic effect. The condition was apparently not of an infectious nature. The various medications and additives commonly found in feeds did not cause the disease.

The accumulation of fluid in the pericardial sac is the characteristic gross lesion of the condition called chick edema or "water belly." As much as 20 ml. of fluid have been collected from the pericardial sac of broilers in advanced stages of the condition. In most cases of field outbreaks of this condition the affected broilers had from 100–500 ml. of liquid in the abdominal cavity (1).

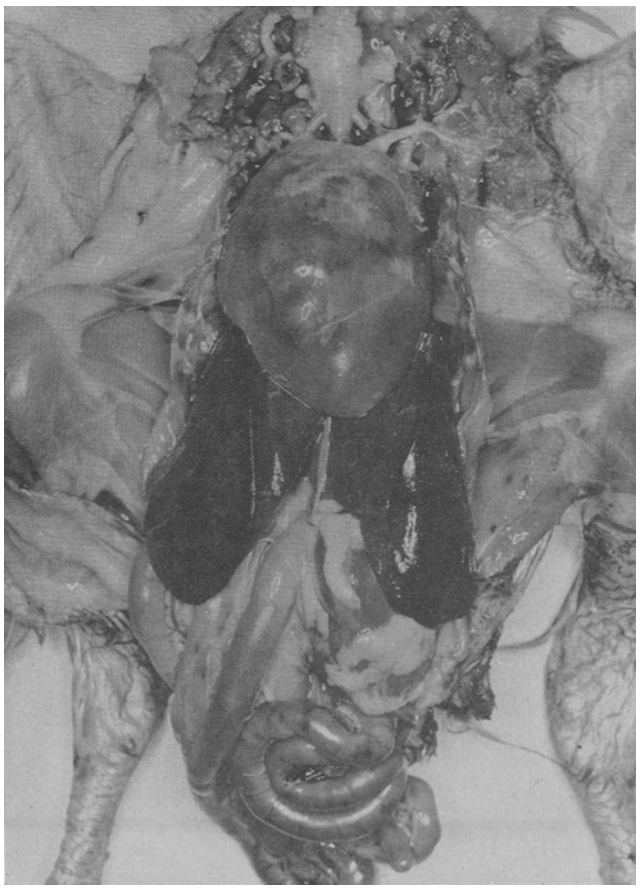

FIG. 1. Hydropericardial condition in broiler suffering from "chick edema."

Edgar (2) and Schmittle (3) described the histopathological picture of the condition in some detail. Liver sections showed small foci of necrosis and degenerating cells. In the early stages the liver lobules were intact, and there was no increase in fibrous tissue.

In the kidney epithelial cells in the proximal convoluted tubules exhibited albuminous degeneration. There was some indication of interstitial edema.

The adrenal gland had petechiae and ecchymoses as well as edema in the interstitial spaces. Some parenchymatous cells had undergone albuminous degeneration.

In the more advanced stages of this condition lesions differed mainly in degree from the early stages. The heart also had focal subepicardial hemorrhages and lymphocytic infiltrations. The epicardium was separated from the myocardium by the hemorrhage and edema present. In the later stages the tips of the villi in the duodenum showed necrosis, but the deeper layer of mucous membrane and the wall were unchanged.

In the terminal stages of the condition the liver cells were nearly all destroyed and necrosis of entire lobules had occurred.

SANGER (1) reviewed a number of conditions in the chick that resembled superficially the histopathological picture found in the chicks receiving 2–5% of the fat containing the toxic principle. Avian infectious hepatitis (4, 5, 6), salt poisoning (7, 8, 9), *vitamin E deficiency* (1), *and some chemical and* plant poisons fall into this category. However none of these conditions show the extensive liver necrosis found in this case.

Vitamin E when given in the feed or injected did not prevent the appearance of symptoms or reverse the condition once they became evident (1, 10, 11, 12).

Vitamin E cannot be excluded completely from this picture as Machlin *et al.* (13) found that a diet designed by them for vitamin E studies produced a quick response to the toxic principle. Brew *et al.* (14) used a modified Machlin diet without a depletion period and found that two weeks were ample for symptoms to appear. This meant a considerable saving of the tedious labor of preparing the toxic concentrates could be achieved.

As a result of intensive work in a number of industrial, government, and university laboratories it was found that certain shipments of animal fat from specific sources caused the symptoms noted above. The toxicity was found to reside in the nonsaponifiable portion of the fat.

As a result of the preliminary laboratory and field work on the edema problem the following conclusions were drawn: a) the malady was not infectious and not of bacterial origin; b) commonly found medications and additives did not reproduce the condition when fed in the diet; c) certain shipments of fat from specific sources could reproduce the condition and the toxicity was caused by some unknown ingredient in the nonsaponifiable portion of the fat; d) the fats *per se* were not the causative agent as feeding of fatty acids prepared from samples of fats containing the toxic principle did not cause the edema.

As quickly as it was established that the toxicity was in the nonsaponifiable portion of the fats, a number of laboratories started attempts to fractionate this part of the fat. Column chromatography on alumina, silicic acid, and silica gel soon established that an abnormally high hydrocarbon content was present in the nonsaponifiables of the fat which causes the trouble.

In a report to the Nutrition Council of the American Feed Manufacturers Association (11) a number of possible contaminants of fat were discussed, and the results of feeding trials of these materials were given. Some of the compounds screened were selenium and nickel, petroleum compounds including tetraethyl lead, polymerized vegetable oils, $\triangle^{-3,5}$ cholestadiene, organic aldehydes of an aliphatic nature, and nitrogen-containing compounds. In general, most of these feeding tests were based on either preliminary analyses of the fats containing the toxic principle or the relative probability of the fat becoming contaminated with such materials.

For instance, slightly greater than normal amounts of nitrogen were found in the nonsaponifiable portion of these fats. This led to the testing of aromatic and aliphatic nitriles, and pyrrol and pyrrolidone by feeding trials.

In the same way preliminary fractionations of the nonsaponifiable material indicated that large amounts of hydrocarbons were present. This led to the obvious inference of contamination with petroleum-based materials. As a result of the analysis of the hydrocarbon fraction $\triangle^{-3,5}$ cholestadiene was found to be a major constituent and was tested by feeding trials. None of the various materials tested gave symptoms identical to those caused by fats containing the toxic principle.

In the same report to the Nutrition Council the following materials were discussed: alpha-tocopherol both fed and injected; antioxidants and the essential fatty acids—linoleic and linolenic. These were not screened for possible toxicity but for curative effects once the birds began to exhibit the edema syndrome.

At first, reports were made that certain antioxidants showed curative powers, but eventually it was shown that none of the compounds tried, whether antioxidant, vitamin, or essential fatty acids, had a curative effect once the chick began to show the symptoms of edema.

Once it had been established that the toxic principle was present in the nonsaponifiable portion of the fat, the research efforts were directed toward two objectives: a) to develop a screening test applicable at the mill level to detect suspect fats; and b) to isolate and identify the toxic material or materials.

The fats containing the toxic principle characteristically had an ultraviolet absorption much higher than normal wholesome animal fats. This high ultraviolet absorption was greatly magnified when the nonsaponifiable portion was isolated and the absorption of this material followed in the region of 220 mμ to 300 mμ.

Figure 2 shows the absorption curve of $\triangle$-3, 5 cholestadiene which is typical of a conjugated diene structure. This absorption curve is similar in shape to the absorption curve of nonsaponifiable material isolated from fats containing the toxicity. The use of ultraviolet absorption as a method of screening suspect fats was not practical at the mill level because of the cost and complexity of the equipment needed.

THE USE of quantitative chromatographic separation of the hydrocarbon portion of nonsaponifiable material and the weighing of this portion was also feasible. However the method was time-consuming, which meant that only those plants having facili-

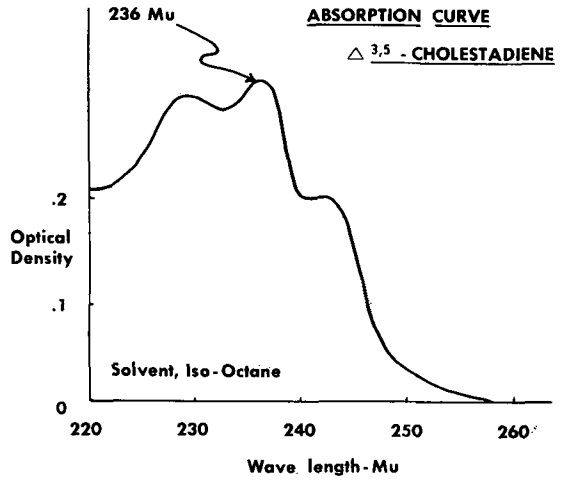

FIG. 2. Ultraviolet absorption curve of 3,5-cholestadiene.

ties to store tank cars of fat some little time could use the method (14).

A simpler chromatographic method in use in a number of plants now consists of passing a weighed amount of fat in Skellysolve F through a fixed amount of alumina. The colorless filtrate contains most of the hydrocarbons but no cholesterol. An aliquot of this filtrate is evaporated to dryness, dissolved in chloroform, and subjected to a Liebermann-Burchard reaction. At the mill level the green color produced is compared to a nickel sulfate standard, and all fats in which the color produced is darker than the standard are biologically tested before use in feed manufacture.

It must be emphasized however that the hydrocarbons isolated by either chromatographic technique are nontoxic in themselves. It is a "guilt by association" sort of thing as large amounts of hydrocarbon have been found in fats containing the toxic principle from the field. When the chromatographic procedures are applied to hydrolyzed fats, the hydrocarbon values found are always somewhat higher than for animal fats. The extent of these differences is shown in Table I. Biological testing has shown that the hydrolysis procedure as normally applied does not produce the toxic factor.

Research aimed at the second objective of identifying the toxic principle is still going forward at this time, and much progress has been made. A recent paper by Brew *et al.* (14) states that chromatographic procedures have been studied which are capable of concentrating the toxic factor at least 3,200 times. At a level of less than 2 p.p.m. in the diet the toxic factor is detectable by biological tests.

Figure 3 shows the preliminary chromatographic procedure used by Brew and co-workers in the separation of toxic fractions from the nonsaponifiable portion of fats containing the toxicity. The 0.75% ether fractions from Step II and Step III when fed at a level of 0.0125 and 0.005%, respectively, caused 100% hydropericardium and ascites symptoms and mortalities of 100 and 88%, respectively, in chicks during the test period of two weeks.

TABLE I
Hydrocarbon Content of Various Fats

Material	Diene hydrocarbon	Total hydrocarbon	Optical[a] density
	%	%	
Non-toxic fats	0.001–0.003	0.05–0.15	0 –0.06
Hydrolyzed fats	0.02 –0.10	0.10–0.50	0.01–0.25
Toxic fats	0.70 –2.0	1.7 –6.6	0.6 –1.9

[a] 1cm., 625 mμ.

Several modifications of the basic chromatographic method have resulted in more nearly quantitative recovery of the toxic fraction. One modification consists of removing all material forming urea adducts before further fractionation on silica gel. This procedure will remove 10% of a nontoxic material from the active fractions.

The use of molecular distillation has resulted in enriched fractions but no pure material of a toxic nature. When the whole fat is distilled, the greatest activity is in the same cuts as the sterols, that is, early but not the first cut (14). When nonsaponifiable material is molecularly distilled, the greatest activity is found in the still residue. This would indicate a fairly large molecule, and this is verified by mass spectrographic data which indicate a molecular weight of 366 for the major fraction. The ebullioscopic method gives a molecular weight of 300 ± 15. No explanation can be given at the present time for the difference in molecular weights by the two determinations.

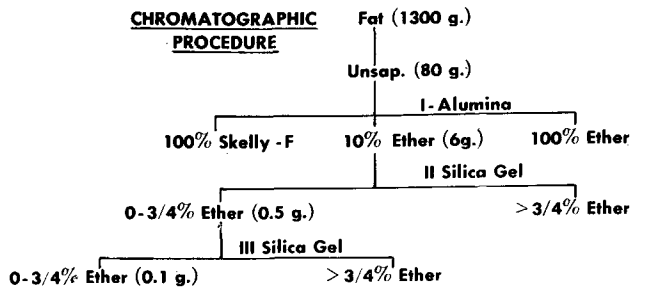

FIG. 3. Preliminary chromatographic separation procedure used in isolating toxic principle from certain processed fats.

FURTHER treatment of highly concentrated chromatographic fractions with Girard T reagent, followed by phthalic anhydride separation, indicated that the activity could be concentrated into the nonketonic, nonalcoholic fraction. This comprises about 65% of the most highly concentrated chromatographic fraction.

Infrared absorption curves of consecutive chromatographic fractions (Figure 4) show bands near 6.2, 12.2, 12.8, and 13.5 μ. These bands are suggestive of a hydrocarbon similar to 1,2-disubstituted naphthalenes but are not identical to any compounds of this type for which curves are available. Fractions A and C are less toxic than B.

These particular fractions were not clean-cut, and the spectra shown no doubt are a result of a mixture of compounds. Near infrared data indicate methyl groups, probable methyl branching, and internal unsaturation.

The ultraviolet spectrum has a peak at 230 and 255 mμ and a small peak at 323 mμ that is not typical of substituted naphthalenes or phenanthrenes. The ultraviolet spectrum indicates only one aromatic ring in the major component.

The toxic concentrates from chromatography give a weak Liebermann-Burchard test. The color resulting from the Liebermann-Burchard test is known to vary with structural differences.

The refractive index was found to be 1.5429, and the same fraction had an optical rotation of + 15°.

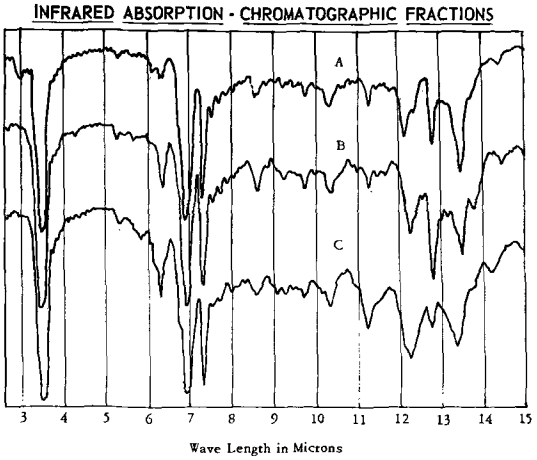

FIG. 4. Infrared absorption curve of toxic principle isolated by chromatographic procedures.

However the concentrate was known not to be a pure compound so these values are only indicative of general structures.

The material toxic to chickens does not show cortical steroid hormone activity. The test animals were adrenalectomized rats, and a modified Kagawa test was used in the assay.

Brew *et al.* (14) concluded on the basis of information available that the toxic material is probably a result of the alteration of cholesterol to a hydrocarbon compound or series of hydrocarbon compounds with some oxidized forms possibly having toxic properties. The molecular weight from mass spectra data indicates that a cholestatriene isomer could possibly be the toxic compound. However further work on the structure of the toxic compound is necessary before a final decision can be made as to the exact structure of the toxic material.

In summary, the following points may be emphasized: a) the toxic material is found only in certain lots of fat subjected to special fat processing operations and fat *per se* is not involved in the toxicity; b) steps have been taken by the feed industry to prevent the incorporation of fats containing the toxic principle into feedstuffs. At the same time research is being carried on to determine the structure of the toxic material so as to enable the feed industry to have a more precise control over fats destined for use in animal feeds. The research work on this problem has been an excellent example of cooperation between industrial, university, government, and private laboratories.

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A Study of the Continuous Production of Mayonnaise

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OVER THE PAST TWO DECADES all aspects of the vegetable oil industry have been transformed slowly from an assembly of batch processes, wherein operator experience played a key role, into a mechanized industry of truly continuous and automatic operation. This trend has been particularly marked in the areas of oil extraction, refining, deodorizing, and in the finished-product lines of margarine manufacture. Mayonnaise production however has been somewhat slower in following this trend although the elements of fully continuous operation have been available for some time.

During the late '30's mayonnaise manufacturers began to use the colloid mill as a means of speeding up the final fixing of the mayonnaise emulsion. Since that time essentially all the major producers have switched to this equipment, and its value is now well established. Even so, the blending of mayonnaise base and its preparation for continuous milling have remained a batch system in most operations throughout the country. This blending is considered an art, jealously protected, but nearly every plant will admit that a good product is made "most of the time" with occasional breakdown of the emulsion into a complete liquid system without apparent reason.

In an attempt to develop a truly continuous plant, from metering the basic raw ingredients to the final milling, a cooperative program has been set up between the Torresdale Company of Philadelphia and the Manton-Gaulin Manufacturing Company of Everett, Mass. This paper should serve to outline the problems and the methods used to establish proper solutions. Basically there are three problems.

First, all materials must be metered accurately in spite of changes in physical properties. Moisture in the air must not affect metering rates of dry ingredients, viscosity must not affect oil flow rate, and, most important, emulsion stiffness must not alter transfer of uniform product-weight from point to point in the process.