pool and was essentially equal in other respects than the processing conditions.

Replacement of the butterfat with vegetable fats did not apparently affect nutritive value in the product investigated here. Bixby and coworkers (*3*) stated that removing the butterfat and replacing it with corn oil depressed the growth rate of rats, but no data were given or references cited on this point.

The results of the current study differ from those of Freeman and Ivy (*7*), both with respect to the weight gains of rats fed the vegetable fat milk product compared to those fed conventional evaporated milk and with respect to the occurrence of diarrhea. The three products used in the present study had equal fat contents, 7.90% before dilution, whereas the filled milk used by Freeman and Ivy (*7*) contained only 6% fat as compared to the 7.74% fat in their evaporated milk, introducing a substantial caloric difference that might have influenced the deposition of body fat and consequently the weight gains. Nevertheless, these authors associated the relatively poorer growth in their filled milk group in the second half of the experimental period with the high incidence of loose stools, and suggested that failure of the intestinal tract to adapt to the vegetable fat might account for the observations.

With three generations of rats in this study, the only difficulty in adapting to the diets was among the first generation animals that had been weaned to a dry ration before starting on the milk diets, and the incidence of loose stools was higher among the rats getting butterfat in the milk than among those getting the vegetable fat. The condition improved markedly in all groups after the first few weeks of the experiment. The litters of the second and third generations had the respective milk diets available *ad libitum* during the nursing period, and made the transition uneventfully from mother's milk to the respective diet milk.

These results are not necessarily inconsistent with those of other workers. The data showing a loss of protein efficiency in evaporated milks indicate only about 10% loss, and this is manifested only under critical conditions in the rat, when the requirement for cystine, cysteine, and methionine becomes the limiting factor (*9*). Under the *ad libitum* feeding conditions in the present experiment, differences in protein nutritive value were not reflected significantly in the growth responses of the rats.

The greater physiological stress imposed on the females by repeated pregnancy and lactation provides a more critical measure of the nutritional adequacy of the diets. The conventionally processed milk appeared to be nutritionally less adequate than the other two milks, while the vegetable fat milk product appeared to be the most adequate, although statistical study of the differences with respect to the individual criteria of conception rates, weaning rates, weaning weights, and weight gains does not show consistently significant differences.

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Received for review July 15, 1957. Accepted October 7, 1957. Investigation supported by Foremost Research Foundation, San Francisco, Calif.

TOXICITY IN MEAT SCRAP

Effect of Trichloroethylene-Extracted Meat Scrap on Young Cattle

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Calves fed meat scrap produced by azeotropic extraction-dehydration with trichloroethylene developed a moderate to severe thrombocytopenia and relative lymphocytosis and, in one instance, leucopenia. These effects are similar to those induced in young cattle by feeding certain specimens of trichloroethylene-extracted soybean oil meal. Development of toxic properties in cattle feeds processed with trichloroethylene is not restricted to soybeans.

THE EXTRACTION of oil-bearing seeds, particularly of soybeans, with trichloroethylene has been initiated at various times in several countries, but the attractive features (*16*) of this process have been overshadowed by the almost invariable occurrence of cases of fatal aplastic anemia when the extracted residues were fed to cattle (*12*). For this reason, commercial production of trichloroethylene-extracted soybean oil meal has apparently been abandoned,

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for the time being.

The chemical nature of the toxic factor in trichloroethylene-extracted soybean oil meal and the conditions with respect to composition of the soybeans and processing which lead to the production of highly toxic or relatively nontoxic specimens are unknown (*10*). No explanation is at hand why the bovine (*9*) and equine species (*8*) are highly susceptible to the toxic factor, whereas many other species (*7*) are relatively resistant. Until such information is available and the safety of trichloroethylene-extracted products can be ascertained by simple tests, the application of trichloroethylene to the processing of food and feed entails potential hazards.

In conjunction with other studies on the toxic factor in trichloroethylene-extracted soybean oil meal, the biological effects of a trichloroethylene-extracted product of animal origin were investigated with a highly susceptible species. This paper summarizes the effects of feeding trichloroethylene-extracted meat scrap on the bovine. While meat scrap is not widely used in rations for cattle, it has been recommended (*6*) as a suitable substitute for other protein concentrates, and, if commercially produced, it would no doubt be used in such rations, inadvertently or otherwise.

Experimental

Meat Scrap. The commercial meat scrap—a dry rendered product—was purchased on the open market. A representative specimen contained 53.7% crude protein, 26.6% ash, and 7.8% moisture.

The trichloroethylene-extracted meat scrap was prepared for this work by batchwise azeotropic extraction-dehydration (*4*) of a mixture containing 22% pork spleens, 22% beef spleens, and 56% horse meat. During the extraction-dehydration of the meat mixture, the temperature of the vapor from the cooker varied from 165° to 185° F. The total time required for filling the cooker and for cooking varies from $3^1/_2$ to $5^3/_4$ hours for different batches weighing from 4100 to 7000 pounds of meat mixture. The solvent was removed from the drained solids by application of indirect heat for about 2 hours in a rotating drum at an absolute pressure of about 25 inches of mercury. A direct steam sparge was then used for an additional 30 minutes under an absolute pressure of about 10 inches of mercury. During the solvent removal, the temperature of the solids rose from 106° to 160° F. and finally to 210° F. The residues from the four batches were then thoroughly mixed and blended with 100 pounds of bone meal per 185 pounds of meat product to yield the material which was used for feeding. It contained 57.8% crude protein, 32% ash, and 4.4% moisture.

Feeding Trials. Two consecutive feeding trials were made with female Holstein calves, weighing initially about 100 pounds. Throughout the trial, the calves were housed in a stable; the meat products were fed after an initial period of observation.

In the first trial (group I), two calves (1146 and 1148) after a preliminary 5-week period of observation, received the trichloroethylene-extracted meat scrap, and one calf (1147) received the commercial product. The meat products were fed initially at a level of 25% of a concentrate mixture which, in addition, contained 40% of ground corn, 24% ground oats, 10% wheat bran, and 1% sodium chloride fortified with trace minerals. Prophylactic doses of vitamin D were given intermittently. In addition, the calves received initially good quality alfalfa and brome grass hay *ad libitum*. After 3 months, hay of poorer quality was offered in restricted amounts and the meat scrap was gradually increased to 50% of the concentrate at the expense of corn.

For 3 weeks, molasses was added to the concentrate mixture in an attempt to increase palatability and reduce its dusty characteristics. By these devices, the intake of meat product reached about 3.5 to 4 pounds per day. During 37 weeks of feeding meat scrap, each calf consumed an estimated 1600 pounds of mixed concentrate containing about 500 pounds of meat scrap. After 42 weeks of the trial, all three animals were fed a normal dairy ration without meat scrap. Two calves were slaughtered $2^1/_2$ weeks later, while one calf (1146), which had developed a severe thrombocytopenia and leucopenia, was kept under observation, outdoors, for 40 weeks longer.

In the second trial (group II), two calves (1202 and 1203) were fed trichloroethylene-extracted meat scrap and one calf (1204) received the commercial product. Throughout this trial, the meat product was suspended twice daily in fresh cow's milk. This mixture was readily consumed within a few minutes. The amount of meat product thus fed per animal per day was 1 pound during the first 26 days, 2 pounds during the next 41 days, and 3 pounds during the remainder of the feeding trial which lasted 35 weeks. During the first 120 days of the trial, the amount of milk fed to each calf per day was about 10% of the body weight; later this was decreased to about 7.5%. Alfalfa hay was fed *ad libitum* and mineralized sodium chloride was available to the calves. Prophylactic doses of vitamin D were given intermittently. The total amount of meat scrap consumed by the calves was 600 pounds for animals 1202 and 1204, and 595 pounds for animal 1203. At the conclusion of the feeding trial with the meat products, all three calves were fed a normal dairy ration, without meat scrap, and kept under observation, outdoors, for 15 weeks longer, when they were slaughtered.

Hematologic Studies. Blood was removed from a jugular vein at frequent intervals for determinations, by standard methods (*2*), of hemoglobin, hematocrit, erythrocytes, thrombocytes, and total and differential leucocyte counts. In addition, stained smears were prepared—at intervals of about 1 week—of bone marrow biopsy specimens removed from the sternubra while the calves were small, and later from the tuber ischii or the tuber coxae. Daily clinical observations were also made, and, at slaughter, the animals were carefully inspected for presence of lesions.

Results

Clinical Observations. All animals made good weight gains, as shown in Table I. Except as noted below, they appeared to be normal. During 3 days in the 15th week of the trial, all three calves in group I had elevated rectal temperatures. Concurrently, calf 1203 developed a leucopenia followed by a relative neutrophilia. A slight diarrhea of about 12 hours' duration was observed in two calves, but all animals continued to eat normally. These signs are not unlike those seen in mild cases of leptospirosis. However, in serological tests performed 5 weeks later with live antigens of the four most common types of *Leptospira* found in cattle, animal 1146 had a titer of 1 to 10 to *L. pomona* and to *L. icterohemorrhagiae;* animal 1148 had a titer of 1 to 10 to *L. icterohemorrhagiae*. Animal 1147 gave a negative test. Titers of less than 1 to 100 are generally not regarded as indicative of bovine leptospirosis (*15*).

During the 39th week, animal 1146 developed lameness and extensive swelling of the right hind leg over the hip region shortly after it had been in estrus. This condition gradually disappeared without treatment. During the 52nd week, 11 weeks after feeding of meat scrap had been discontinued, animal 1146 had a 4-day period of pyrexia, anorexia, depression, slight epistaxis, and petechiation of the vaginal mucosa was observed. At the same time, there was a sharp drop in hemoglobin from 11.3 to 9.0 grams per 100 ml., a concomitant decrease in the red cell count and hematocrit, and the leucocyte count decreased from 6000 per cu. mm. on March 3, 1955, to 1800 per cu. mm. on March 11. A bone marrow biopsy specimen removed on March 17 showed a great abundance of immature forms of the erthyroid series, presumably in response to preceding hemorrhage.

Animal 1203 had a mild case of pneu-

monia in the 12th week, and, in the 21st week all three animals of group II showed symptoms of pneumonia. In each instance, the animals responded quickly to treatment with streptomycin. Post mortem examination at slaughter revealed no lesions in any of the animals.

Hematologic Observations. Except as noted above, there was no difference in hemoglobin concentration, erythrocyte count, or hematocrit of the calves receiving the two types of meat product. A summary of the mean values, calculated for 10-week periods, of thrombocytes, and per cent lymphocytes for each calf is given in Table II. During the first 10 weeks, all values were within the range of those usually observed for calves of this age. For calves 1147 and 1202, fed commercial meat scrap, values remained in the normal range throughout the trial. During the third 10-week interval, and thereafter, calf 1146 had a definite leucopenia and developed a relative lymphocytosis which became particularly pronounced during the fourth 10-week interval. These conditions persisted in this animal for 30 weeks after feeding of trichloroethylene-extracted meat scrap had ceased, and in the 10th week of this period a leucocyte count of 1800 was recorded. In the other three calves fed trichloroethylene-extracted meat scrap, the leucocyte counts remained in the normal range, but in calf 1202 there was also a definite tendency toward the establishment of a relative lymphocytosis, particularly in the third and fourth 10-week intervals of the trial.

The thrombocyte counts of the calves fed the commercial meat scrap showed the gradual decrease usually observed with increasing age of young cattle (*11*). As illustrated in Figures 1 and 2, and summarized by periods in Table II, the thrombocyte counts of the calves fed the trichloroethylene-extracted meat scrap were distinctly lower, after the first 10 weeks of feeding. In calves 1202 and 1203, thrombocyte counts reached the normal range again in the third 10-week interval, whereas in calves 1148 and particularly 1146 they were below normal throughout the trial. As shown in Figure 1, a severe thrombocytopenia in calf 1146 persisted long after feeding of trichloroethylene-extracted meat scrap had ceased, and this animal made only a temporary recovery in this respect. The last thrombocyte count, 220,000, was taken 38 weeks after feeding of trichloroethylene-extracted meat scrap was discontinued.

Table I. Weight Gains of Calves

Group	Animal No.	Type of Meat Scrap	Period, Days	Weight Gain, Lb. Total	Weight Gain, Lb. Daily
I	1147	Commercial	260	500	1.92
I	1146	TCE-extracted	260	542	2.08
I	1148	TCE-extracted	260	476	1.83
II	1204	Commercial	246	487	1.98
II	1202	TCE-extracted	246	456	1.85
II	1203	TCE-extracted	246	500	2.03

Discussion

The most sensitive criteria thus far found for detection of the toxic factor in trichloroethylene-extracted soybean oil meal are thrombocytopenia and the establishment of a relative lymphocytosis (*7*). The former was observed in all four calves fed trichloroethylene-extracted meat scrap, and the latter in two of the four animals receiving this product.

The present results, considered in the light of the authors' experience (*10, 11*) with trichloroethylene-extracted soybean oil meal fed continuously to calves of similar age, indicate that feeding of trichloroethylene-extracted meat scrap can induce a blood dyscrasia similar in type and severity to that produced by prolonged feeding of low levels (about 1 pound per day) of a relatively toxic specimen of trichloroethylene-extracted soybean oil meal, or by feeding of higher levels (about 3 pounds per day) of specimens of trichloroethylene-extracted soybean oil meal of a lower degree of toxicity.

These results, therefore, indicate that the trichloroethylene-extracted meat scrap had similar biological effects on the bovine as the toxic factor in trichloroethylene-extracted soybean oil meal. However, the amount of this factor present in the meat product evidently was low, because in the young calf the consumption of as little as 0.025 pound per day per 100 pounds of body weight of a highly toxic specimen of trichloroethylene-extracted soybean oil meal produces a definite blood dyscrasia in about 30 days, and even the effects of 0.01 pound can be detected in some individuals (*7*).

Evidence for toxic properties has been obtained with trichloroethylene-extracted

Table II. Mean Thrombocyte, Leucocyte, and Lymphocyte Counts of Calves Fed Meat Scrap

Type of Meat Scrap	Animal No.	Items Compared	Time Interval, Weeks: To 10	11–20	21–30	31–40
Commercial	1147	Thrombocytes[a]	857 ± 52.9[b] (12)[c]	623 ± 20.4 (7)	589 ± 22.6 (15)	550 ± 41.0 (9)
		Leucocytes[a]	10.5 ± 1.3 (12)	10.6 ± 00.63 (7)	10.7 ± 0.36 (15)	10.2 ± 1.4 (8)
		Lymphocytes[d]	58.9 + 2.7 (12)	71.4 + 2.9 (7)	61.9 + 2.4 (15)	68.6 + 1.6 (9)
	1204	Thrombocytes	667 ± 25.9 (13)	610 ± 26.1 (10)	509 ± 18.3 (11)	495 ± 24.5 (8)
		Leucocytes	8.69 ± 0.42 (13)	9.17 ± 0.57 (10)	9.24 ± 0.57 (10)	9.79 ± 0.99 (8)
		Lymphocytes	62.4 ± 2.8 (13)	64.0 ± 1.6 (10)	59.8 ± 2.2 (11)	58.7 ± 4.0 (8)
Trichloroethylene-extracted	1146	Thrombocytes	879 ± 65.7 (12)	542 ± 42.2 (7)	237 ± 23.8 (15)	131 ± 7.2 (9)
		Leucocytes	12.1 ± 0.85 (12)	8.9 ± 0.42 (7)	4.7 ± 0.36 (15)	4.5 ± 0.31 (9)
		Lymphocytes	55.2 ± 3.2 (12)	69.0 ± 1.7 (7)	73.3 ± 2.3 (15)	90.9 ± 1.4 (9)
	1148	Thrombocytes	817 ± 48.0 (12)	555 ± 18.3 (7)	321 ± 17.4 (15)	357 ± 20.6 (9)
		Leucocytes	13.9 ± 0.97 (12)	11.9 ± 0.67 (7)	10.2 ± 0.57 (15)	9.3 ± 0.67 (9)
		Lymphocytes	42.7 ± 3.5 (12)	55.3 ± 1.6 (7)	55.0 ± 2.1 (15)	56.1 ± 2.1 (9)
	1202	Thrombocytes	659 ± 71.1 (17)	442 ± 20.1 (10)	379 ± 27.3 (11)	478 ± 31.4 (8)
		Leucocytes	9.74 ± 0.49 (17)	11.75 ± 0.79 (10)	8.64 ± 0.82 (10)	6.50 ± 0.54 (8)
		Lymphocytes	69.8 ± 3.4 (17)	73.4 ± 2.6 (10)	77.4 ± 1.5 (11)	76.0 ± 1.1 (8)
	1203	Thrombocytes	644 ± 33.5 (16)	465 ± 24.6 (10)	363 ± 20.0 (11)	504 ± 36.9 (8)
		Leucocytes	9.93 ± 0.50 (16)	9.87 ± 0.62 (10)	8.16 ± 0.87 (10)	9.34 ± 0.49 (8)
		Lymphocytes	67.8 ± 2.3 (16)	69.3 ± 3.3 (10)	72.1 ± 2.6 (11)	61.8 ± 3.5 (8)

[a] Thrombocytes and leucocytes, thousands/cu. mm. blood.
[b] Standard error of mean.
[c] Number of determinations.
[d] Lymphocytes, per cent of total leucocyte count.

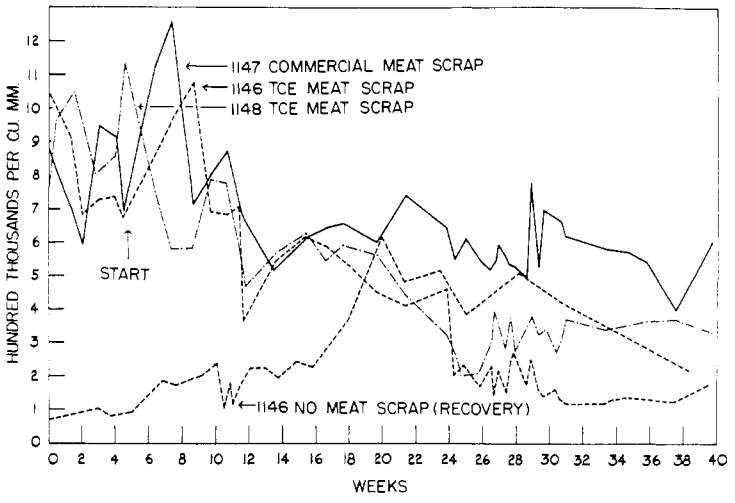

Figure 1. Thrombocyte counts of a calf (1147) fed commercial meat scrap and of two calves (1146 and 1148) fed trichloroethylene-extracted meat scrap

1146, no meat scrap (recovery), represents a period of 40 weeks after feeding of meat scrap to calf 1146 had ceased

product of animal origin, the composition of which is in many respects entirely different from that of soybeans. Moreover, this product was produced under conditions of processing which are different from those used in the manufacture of trichloroethylene-extracted soybean oil meal. Toxic products may therefore be formed from materials other than soybeans and under conditions not restricted to those associated with the processing of soybeans. As trichloroethylene itself is relatively innocuous to the calf (*9*) and can be metabolized by this species (*13, 14*), the present observations strengthen the view that interaction of trichloroethylene or one of its decomposition products and some component present in certain natural products causes the formation of a compound which can induce aplastic anemia in susceptible species. When fed to swine (*3*) and chickens (*1*), trichloroethylene-extracted meat scrap produced no evidence of toxicity. To what extent the formation of such a toxic product would occur under different conditions of processing of various other raw materials can, of course, not be predicted, but the possibility that this can happen should not be overlooked.

After this paper had been accepted for publication, McKinney (*5*) reported that oral administration of *S*-(dichlorovinyl)-L-cysteine produces a typical syndrome of aplastic anemia in calves. It is a matter of conjecture whether the symptoms of toxicity reported in this paper are due to a product of interaction of trichloroethylene and sulfhydryl compounds in the processed meat.

Acknowledgment

The supply of trichloroethylene-extracted meat scrap was from E. I. du Pont de Nemours & Co., Inc., Wilmington, Del., whose assistance is gratefully acknowledged. The authors are indebted to J. J. Hargarten of the above company for communicating details of conditions under which meat scrap was processed with trichloroethylene. Fern W. Bates gave excellent technical assistance.

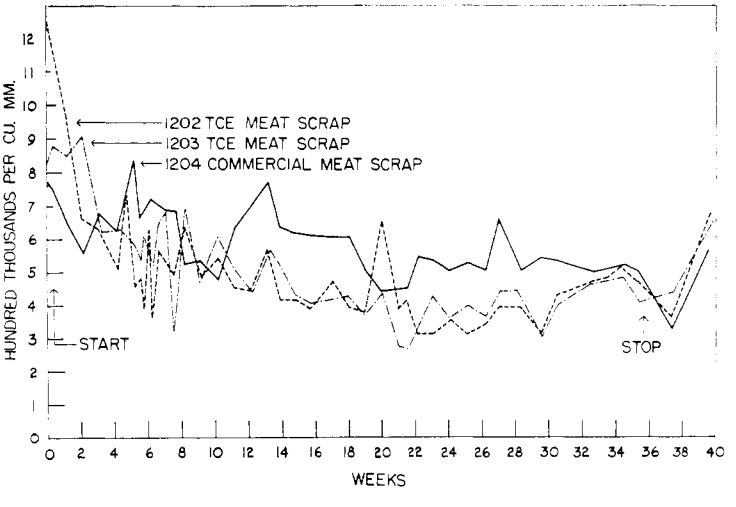

Figure 2. Thrombocyte counts of a calf (1204) fed commercial meat scrap and of two calves (1202 and 1203) fed trichloroethylene-extracted meat scrap

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Received for review January 5, 1957. Accepted March 17, 1957. Paper 3712, Scientific Journal Series, Minnesota Agricultural Experiment Station. Paper XIV of a series entitled Studies on Trichloroethylene-Extracted Feeds. Supported in part by funds from E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. *Address correspondence to M. O. Schultze, Department of Agricultural Biochemistry, Institute of Agriculture, University of Minnesota, St. Paul 1, Minn.*