tary inhibiting effect of 2-PAM. If such an interpretation is correct, it seems logical to assume that 2 aspects of activity of 2-PAM were demonstrated in the preparation described by line 5 of Table I. First, TEPP-inactivated ChE was reactivated and subsequently it was inhibited by the oxime itself.

Conclusion. 2-PAM in a high concentration *in vitro* had a dual effect on motor endplate ChE previously inactivated with TEPP. The oxime reactivated the enzyme and, if allowed to remain in contact with the preparation, inhibited the reactivated enzyme. The effect of 2-PAM was reversible and after the removal of 2-PAM no protection to ChE from the later exposure to TEPP was demonstrated.

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Relationship of Dietary Dienoic Acid Content to That in Mouse Carcass Fat.* (23654)

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The investigation here reported was suggested by results obtained in a previous study (1) that showed an increase in carcass fat dienoic acids of animals fed rigorously extracted soybean oil meal. The results of the study indicated that the increased carcass fat dienoic acids were not the result of residual dienoic acids in the meal. Hence, work attempting to isolate the causative factor was initiated. The fractionation work revealed that the ether-extracted soybean meal contained sufficient dienoic acids, which could be removed by methanol extraction, to account for the increased carcass fat levels of these acids. During these experiments it became necessary to investigate the nature of the response curve of carcass fat dienoic acids to dietary dienoic acids, since little information is available about the quantitative aspects of this relationship especially at low dietary levels.

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Methods. Weanling albino mice obtained from the North Carolina State Laboratory of Hygiene were used in these studies. They were housed individually on wire screens and received *ad libitum* an egg albumin-starch purified diet similar to diet 13 of the previous studies(1). Each animal was parenterally given 0.01 mg biotin twice weekly. The various adjuncts that were tested for their capacity to alter the carcass fat dienoic acid content were added at the expense of starch. Each experiment was of 3 weeks duration with the average of 6 replications reported. At the completion of the experiment the mice were gassed, skinned, decapitated, and the entire gastrointestinal tract removed. The mouse carcass was then placed into a 30-ml beaker, minced with scissors and dehydrated by freeze drying. The dry carcass was ground with about 1 g of Super-Cel and about 2 g of anhydrous sodium sulfate. This mixture was placed into a coarse alundum crucible and extracted with anhydrous ether for 12-18 hrs in a Pickel extractor. After evaporation of the

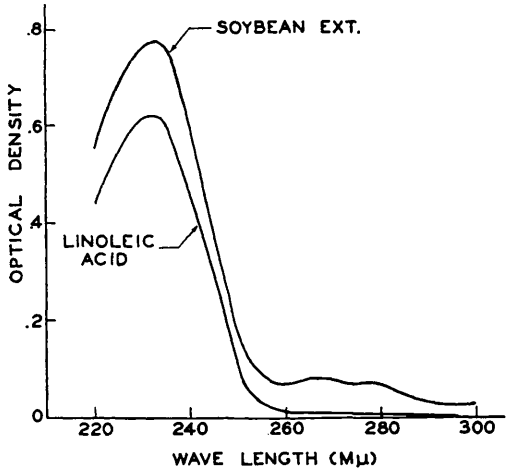

FIG. 1. Absorption spectrum of isomerized fatty acids from soybean extract and linoleic acid at concentrations of 18 and 7 mg/l, respectively.

ether, the polyunsaturated acids in the carcass fat were determined by alkaline isomerization(2). Soybean meal extracts were prepared in the following manner: Commercial solvent-extracted soybean meal was continuously extracted with ether for 48 hrs. The ether extracted meal was then continuously extracted with methanol at 65°C for 48 hrs, and the methanol extract dried on starch after removal of the solvent. An ether-soluble portion of the methanol extract was prepared by extracting the methanol extract, after adjusting the pH to 4, with ether in a continuous liquid-liquid extractor for 48 hrs.

Results. 1. *Factor studies.* Fractionation work aimed toward isolation of the factor suggested by previous work revealed that the factor could be extracted from the meal with methanol, and further that continuous ether extraction of the methanol-soluble portion removed all the activity. Since these results would be consistent with the presence of residual dienoic acids in the ether extracted soybean meal, this possibility was investigated more rigorously than had been done in the previous studies(1).

The ether-soluble fraction of the methanol extract was saponified with 10% alcoholic potassium hydroxide and the fatty acids obtained divided into 2 portions. One of these was subjected to alkaline isomerization and the adsorption spectrum compared with an authentic sample of linoleic acid. The spectral curve, Fig. 1, of the acids closely resembles that of the isomerized linoleic acid. The two peaks observed in the region of 270 mμ are characteristic of isomerized trienoic acids. Chromatography of the second portion according to the procedure of Simmons and Quackenbush(3) revealed that both dienoic and trienoic acids were present.†

Although previous results(1) indicated that a solvent extract of acid-hydrolyzed soybean meal failed to increase the carcass fat dienoic acids, the detection of polyunsaturated acids in the soybean meal extracts necessitated repetition of this experiment. The lipids from 750 g of ether-extracted soybean meal were obtained by ether and petroleum ether extraction after acid hydrolysis according to the AOAC procedure for the determination of fat in cereal products(4). After removal of the solvents under reduced pressure, the lipid was dissolved in ethanol and dried on starch at room temperature. Contrary to the previous results from this type of preparation(1), the lipids equivalent to 75% soybean meal increased the carcass fat dienoic acids from 2.7% to 6.1%

2. *Response curve studies.* Prior to the detection of the presence of polyunsaturated acids in the soybean meal extracts, attempts were made to develop a quantitative assay for the substance in these extracts that increased the dienoic acid content of carcass fat. In the previous work(1), the greatest relative response was obtained when no dietary fat was added to the basal diet. Therefore, a basal diet without added fat was adopted, and this was supplemented with graded levels of the soybean meal methanol extract. The results of two such experiments, Fig. 2, show that although the lower part of the response curve is linear, at the highest level of soybean meal extract the response curve becomes curvilinear. When the logarithm of intake is plotted against the logarithm of carcass fat dienoic acid level, a straight line, which includes the high level, is obtained.

To further verify the response curve and to

† The authors are indebted to Dr. R. O. Simmons for the chromatographic analysis.

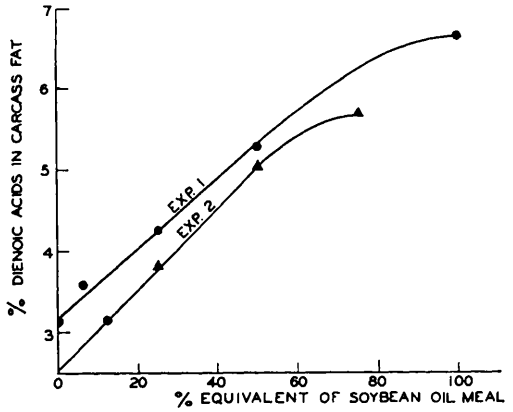

FIG. 2. Effect of feeding methanol extract of soybean meal on dienoic acid content of mouse carcass fat.

extend its range to 400% equivalent soybean meal a third experiment was conducted. The organic solids of the methanol extract as determined by the method of Johnson(5) using a sucrose standard was equivalent to 140 mg/g of soybean meal. Thus, if the extract were fed equivalent to 400% soybean meal, at least 56% of the diet would be composed of extract solids. Since the ether-soluble extract equivalent to 400% soybean meal would contain only 13.6% organic solids, this ether extract was used instead of the crude methanol extract. To serve as a basis of comparison, graded levels of soybean oil also were fed as a source of dietary dienoic acids. As was observed previously, a curvilinear response was obtained, Fig. 3, and a log-log plot resulted in a straight line up to an equivalent of 200% soybean meal and a dietary level of dienoic acids of 0.8% as soybean oil. However, when either 400% soybean-meal equivalent or 1.6% dienoic acids as soybean oil was fed, the carcass fat dienoic acids were distinctly higher than would be expected by extrapolation of the curve.

Discussion. In view of the similarity of the response curves from the ether-extracted soybean-meal fractions to that from soybean oil, the chemical properties of the active principle and the demonstrated presence of dienoic acids in both the ether-extracted soybean meal and its active fractions, it is apparent that the increased carcass fat dienoic acid level obtained by feeding the ether-extracted soybean meal is a result of residual amounts of dienoic acids in the meal. From the response curve of Fig. 3, it can be calculated that the meals or meal extracts should contain approximately 0.4% dienoic acids. This is in good agreement with the 0.4% calculated from feeding the fat-soluble portion of the acid hydrolyzed meal and 0.38% and 0.47% found by analysis of the extract by alkaline isomerization and chromatography, respectively.

In the previous work(1), the lipid fraction of acid hydrolyzed meal failed to produce a response. It is possible that the dienoic acids were oxidized during the course of diet preparation or storage, but there is no evidence at hand to support this.

It is apparent from an examination of the response curves obtained when graded levels of dienoic acids are fed either as soybean oil or soybean meal extracts that up to a level of 0.8% dietary dienoic acids a curvilinear response curve is obtained. Within the range of 0.2% and 0.8% dietary dienoic acids, plotting the logarithm of dietary level against the logarithm of carcass level results in a straight line. The log-log relationship would be particularly useful in the determination of biological availability of dietary dienoic acids. At dietary levels greater than 0.8%, a greater proportion of the ingested dienoic acids is found in the carcass fat.

Ellis *et al.*(6) fed graded levels of cottonseed oil to swine and measured the linoleic acid content of the carcass fat. If these data are graphed, an upward curvilinear response curve is obtained. Since the lowest level of

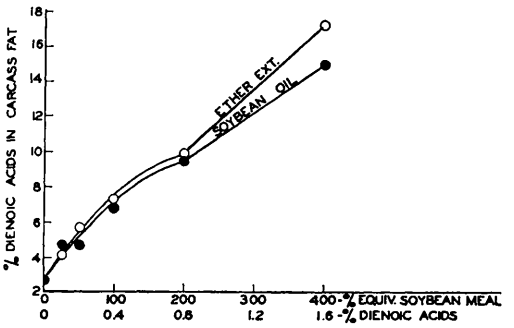

FIG. 3. Effect of feeding ether soluble portion of a methanol extract of soybean meal and dienoic acids as soybean oil on carcass-fat dienoic acid level.

cottonseed oil fed was 3%, these data are not in conflict with the observations reported herein. In fact, the disproportionately increased dienoic acid deposition obtained with the highest level (Fig. 3) suggests a similar upward curvilinear trend. Mead *et al.*(7) have shown that a large proportion of the ingested linoleic acid is rapidly oxidized. Conversely, from the prolonged time required for the depletion of linoleic acid from animal tissues, that deposited is relatively metabolically inert.

It would seem probable that as the dietary level of dienoic acids changes the ratio of the amount oxidized to that deposited also changes. The data presented herein coupled with that of Ellis *et al.*(6) may be interpreted to indicate at least 3 such changes. At dietary intakes up to about 0.2% dienoic acids, the ratio of dienoic acids oxidized to that deposited remains constant. Between about 0.2%-0.8% a larger proportion of the ingested dienoic acids is oxidized, and as the levels increase above 0.8%, an increasingly larger fraction of the ingested dienoic acids is deposited. The foregoing considerations are, of course, largely speculatory and considerably more evidence is required for confirmation.

Summary. (1) Ether extracted soybean oil meal has been found to contain sufficient residual dienoic acids to account for the previously observed increased carcass fat dienoic acids of animals that ingest the meal. (2) Studies were conducted, with mice, on the carcass-fat levels of dienoic acids obtained as the dietary-dienoic-acid levels varied from 0 to 1.6%. Between the dietary levels of 0.1% and 0.8% dienoic acids, a curvilinear response curve was obtained and became linear when the logarithm of dietary intake was plotted against the logarithm of carcass fat level. At a dietary level of 1.6% dienoic acids, a disproportionately higher dienoic acid level was found in the carcass fat.

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Effect of Dibenamine and Pyribenzamine on Hypothermia of Chlorpromazine. (23655)

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Tranquilizing drugs such as reserpine and chlorpromazine injected into an animal are reported to release a variety of substances which independently have a diversity of actions. These substances are 5 hydroxytryptamine(1), some sympathetic neurohumoral agent(2,3,4), and possibly histamine.* All these substances have a common effect which is that of lowering the body temperature(5,6,7,8). The purpose of this study was to determine the effect on body temperature of certain drugs which are known antagonists to these products released by chlorpromazine. The substances used were dibenamine, which antagonizes the edema producing effect of 5-

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