TABLE III

Oil Recovery from Alumina Filter Cake

Adsorbent	Oil recovery			Oil constituents per 100 g. of adsorbent[a]		
	As bleached oil	Extracted from cake with petroleum ether	Total oil recovered	Total in cake	Extracted with petroleum ether	Residual in cake
	g.	g.	%	g.	g.	g.
Alumina[b]	195.4	3.6	99.5	34.7	20.4	14.3
Alumina	195.2	3.1	99.2	36.2	20.3	15.9
Alumina	195.1	3.5	99.3	.....	.....	.....
Natural earth. A.O.C.S.	195.8	3.2	99.5	26.3	13.8	12.5

[a] Determined by method of Rich (5).
[b] Bleached under reduced pressure, 0.5 mm.

alumina is about 35% the weight of the absorbent used. This compares favorably with data obtained with the same method for oil retention on natural earths of commercial origin, namely 26–33% (5). By solvent extraction of spent alumina the oil retention can be reduced to 15%, thus allowing a total recovery of 99.5% of the refined oil as bleached oil.

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Evaluation of Cottonseed Meals Prepared by Extraction with Acetone-Hexane-Water Mixtures[1]

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Eleven cottonseed meals have been prepared by batch extractions of a given lot of cottonseed with various acetone-hexane-water mixtures using several different extraction schedules. These meals, together with eight meals of commercial origin and a commercial soybean meal, have been subjected to chemical evaluation and assayed for protein quality using the growing chick as a test animal. In general the acetone-hexane-water meals were superior to the commercial cottonseed meals for promoting the growth of the chicks and, considering all the meals, a linear correlation was obtained between the logarithm of the weight gains and the available lysine contents of the meals. The free and total gossypol contents of the cottonseed meals appeared to have little or no influence on the growth rates.

UNDUE APPLICATION OF HEAT during the processing of cottonseed has an adverse effect upon the nutritive quality of the meal proteins (1,2). This has been attributed to: a) the destruction of part of the lysine of the seed proteins (3); b) the binding of the *epsilon* amino groups of a portion of the lysine by gossypol and other meal constituents (4,5). Lysine bound in this manner is not available to nonruminant animals (6).

Evidence developed by Frampton *et al.* (7) indicates that the variation in nutritive quality for broilers noted among commercial cottonseed meals is largely accounted for by variations in the lysine contents of the meals, and that the influence of the gossypol contents of the meals is too small to be statistically measured. While the effects of gossypol and gossypol derivatives on the growth of broilers may be subject to question, it has been established that the presence of these compounds is undesirable from other nutritional aspects. For example, these substances are responsible for some of the abnormalities that occur in stored shell eggs produced by hens fed cottonseed meals (8). Moreover, gossypol and gossypol derivatives may be implicated in the mortalities that occur among swine receiving diets containing certain cottonseed meals (9).

To achieve the desired end of obtaining cottonseed meals low in gossypol without application of heat, King, Kuck, and Frampton (10) have proposed the use of a solvent mixture composed of acetone, hexane, and water (A:H:W; proportions by volume, 53:44:3) for the extraction of raw (or mildly-treated) cottonseed flakes. Meals prepared using this solvent proved to be low in gossypol and high in available lysine. (The term "available lysine" refers to the meal lysine having free *epsilon* amino groups as determined (13) by use of 2,4-dinitrofluorobenzene.) Preliminary feeding tests indicated that they were superior to conventional cottonseed meals in promoting the growth of young animals.

This report describes further the use of A:H:W solvents for the preparations of cottonseed meals. The extraction variables investigated included the composition of the A:H:W mixtures, the time of contact between the cottonseed flakes and the first portion of solvent, and the total number of solvent passes used. All meals were characterized chemically and tested for their growth-promoting properties using the chick as a test animal. For comparison eight other meals, primarily of commercial origin, were assayed along with the A:H:W meals.

Experimental

Seed and Flakes. The single lot of delinted seed was stored at 60°F., and from time to time portions were hulled and separated using standard mill ma-

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TABLE I

Data on A:H:W Extractions [a]

Extraction number	H_2O in prec. flakes, %	Prec. flakes extracted, kg.	Composition A:H:W solv., %(v/v)	Duration of first pass, hr.	Total No. passes	Total vol. solv. used, liters	Total vol. miscella, liters	Yield of meal, kg.
1	9.70	13.3	52.6:44.4:3.0	3	6	159	.....	8.3
2	9.70	11.9	52.7:44.3:3.0	17	6	158	.....	6.7
3[b]	9.87	18.2	52.6:44.4:3.0	3	14	494	437	10.2
4[c]	8.92	18.9	51.6:45.4:3.0	17	6	212	174	11.1
6[b]	.....	18.2	51.6:45.4:3.0	3.5	6	212	181	11.4
5	.....	19.5	50.8:46.2:3.0	3	13	461	426	11.7
7	.....	20.1	56.7:43.3:0.0	3	6	210	194	11.0
8	10.47	20.0	56.1:42.9:1.0	3	6	212	193	11.0
9	9.81	21.2	55.5:42.5:2.0	3	6	214	180	11.2
10	9.28	19.2	55.0:42.1:2.9	3	6	216	181	11.4
11	9.13	18.9	42.6:56.2:1.2	3	6	210	180	10.7

[a] Unless otherwise stated, the meals from these extractions are designated M-1, M-2, etc.
[b] The meals from Extractions 3 and 6 were thoroughly blended to yield Meal M-3&6; a portion of this mixture was autoclaved as described in the text to yield Meal M-3&6(A).
[c] The flakes used for Extraction 4 were not preconditioned—see text.

TABLE II

Chemical Characteristics of A:H:W Extracted Meals [a]

Meal	Moisture, %	Percentage on a moisture-free basis								Available lysine, g./16 g. N	Nitrogen solubility,[b] %
		Oil	Nitrogen	Gossypol		Total phosphorous	Sugar		Crude fiber		
				Total	Free		Total	Red.			
M-1	10.68	0.58	10.01	0.37	0.032	1.72	7.78	2.73	3.59	3.98	91.2
M-2	6.97	0.30	10.05	0.49	0.054	1.74	7.48	1.02	3.98	4.11	89.2
M-3&6	10.72	0.16	10.33	0.34	0.026	1.76	7.46	2.86	3.85	4.22	89.8
M-3&6(A)	5.17	0.18	10.43	0.30	0.011	1.81	6.71	2.23	3.95	3.49	73.5
M-4	10.21	2.70	9.79	0.62	0.065	1.69	8.19	2.07	3.35	3.86	89.9
M-5	11.32	0.67	10.12	0.39	0.052	1.75	8.46	1.89	3.37	3.98	91.1
M-7	7.03	1.05	9.95	0.58	0.268	1.71	8.44	0.45	3.57	4.30	94.2
M-8	6.66	1.00	10.00	0.71	0.313	1.74	8.06	0.40	3.49	4.32	91.6
M-9	6.76	1.02	10.03	0.62	0.164	1.74	8.47	0.71	3.42	4.36	88.7
M-10	6.84	0.77	10.12	0.49	0.077	1.76	8.35	1.17	3.17	3.98	90.1
M-11	6.47	1.15	9.98	0.67	0.229	1.74	8.39	0.57	3.25	4.02	90.2

[a] See Table I for data on preparation of meals.
[b] Nitrogen solubility measured using 0.02 *N* NaOH.

chinery. The fine portions were discarded and the whole and cracked meats were thoroughly mixed (yield about 30 lb. per 100 lb. seed) and stored in sealed cans at 40°F. until used. No more than two weeks' supply of meats was prepared at a time.

With the exception of Extraction 4 (Table I), all of the meats extracted were preconditioned in the following manner: a) On the first experimental day a weighed portion of the meats was mixed with an amount of water calculated to raise their moisture content to 15%. The moistened meats were stored in a closed container at room temperature. b) About 24 hr. later, the moistened meats were passed through flaking rolls set at 0.003 in. The flakes were allowed to air-dry.

The extraction of the flakes was started after a 24-hr. period of air-drying. The moisture contents of the preconditioned flakes ranged from 9.1 to 10.4%, averaging 9.7%. The meats used in Extraction 4 were flaked and extracted without preconditioning.

Preparation of Meals. All extractions were performed at room temperature in metallic containers, of about 30-gal. capacity, which were equipped with screens to permit draining of the miscella. The solvent to flakes ratio was fixed at about 1.9 l. per kg.; soaking periods (other than the first pass) were approximately 20 min. each, and the slurry was agitated for about 2 min. after the solvent was first added. At the end of each soaking period the miscella was allowed to drain from the flakes for about 30 min. before the next portion of solvent was used.

Initial soaking periods of 3 hr. and 17 hr. were investigated, and extraction schedules of 6 passes and of 13 or 14 passes were employed.

Extractions 1 through 6 (Table I) were carried out using A:H:W solvents which had been reclaimed and adjusted to contain 3% (V/V) water, while the solvents used for Extractions 7 through 11 were prepared by blending the requisite amounts of pure acetone, hexane, and water. After the last portion of the miscella was drained off, the extracted flakes were spread on stainless steel trays and dried in a vacuum oven operated at 110°F. under 18 in. of vacuum. To achieve satisfactory drying in 15 to 24 hr. it was found necessary to pass a slow stream of air through the oven. The dry, hard lumps of meal generally obtained were crushed by use of cracking rolls. The pulverized meals were stored in sealed cans at 40°F. pending analyses and feeding experiments.

Comparison Meals. Meals 106 through 117 had been prepared earlier for use in another study. Meal 106 was prepared on a large scale in the pilot plant by extraction with an acetone-hexane-water mixture (53:44:3); Meals 109, 111, and 112 were prepress solvent extracted meals; while Meals 107, 108, and 110 were screw-press meals. Meal 113 was a portion of Meal 108 which had been degraded in protein quality by heating it in a French Cooker at 275°F. for 1.5 hr.

The soybean oilseed meal (SBOM) was a high-quality commercial product which contained 50% protein.

Chick Feeding Experiments. These were carried out essentially according to Heiman *et al.* (11). Immediately after the chicks (a crossbred broiler strain) were hatched, they were placed on a standardization ration composed of yellow corn meal and fortified with vitamins and minerals. This ration was fed for for a 7-day period to stimulate the utilization of the residual embryonic protein. The chicks used in the test were selected for uniformity at this time. About a third of the chicks were discarded. The remaining chicks were divided into weight classes in which the weights of the individual chicks did not vary over 5 g. Ration groups of 10 chicks each were assembled, an equal number from each weight class being taken to make up each ration group. This technique reduced the individual variation in each experimental lot.

Three ration groups of the 7-day old chicks were placed on each of the various rations. These were

TABLE III

Chemical Characteristics of Comparison Meals

Meal	Moisture, %	Percentage on a moisture-free basis								Available lysine, g./16 g. N	Nitrogen solubility,[a] %
		Oil	Nitrogen	Gossypol		Total phosphorous	Sugar		Crude fiber		
				Total	Free		Total	Red.			
106	11.22	0.48	9.23	0.37	0.035	1.53	6.26	0.82	7.40	3.68	80.1
107	10.05	3.56	7.43	1.20	0.074	1.31	4.79	0.30	14.24	2.97	56.9
108	9.60	4.29	7.29	1.39	0.056	1.42	5.42	0.48	12.64	2.50	35.6
109	10.52	0.74	7.54	1.04	0.061	1.10	6.92	0.42	12.29	3.37	67.5
110	9.19	4.03	7.29	1.38	0.075	1.43	5.88	0.42	11.35	2.85	33.9
111	9.96	1.28	7.45	1.53	0.093	1.41	6.55	0.44	13.08	3.13	60.9
112	10.89	0.50	7.09	0.90	0.036	1.20	6.12	0.35	16.29	3.54	70.9
113	8.93	4.00	7.29	1.05	0.030	1.39	4.08	0.40	14.45	1.58	16.5

[a] Nitrogen solubility measured using 0.02 *N* NaOH.

formulated to supply 12% protein (nitrogen x 6.25); 6% was supplied by the corn and 6% by the oilseed meal.

The suboptimum protein level of 12% was maintained so that small differences in the protein quality of the several supplements would be exaggerated.

The composition of a typical ration, in lb. per 100 lb. was: yellow corn meal, 65.0; cottonseed meal, 12.8; starch, 9.0; sugar, 9.1; dicalcium phosphate, 2.0; oyster shell flour, 1.0; salt, 0.5; plus 0.6 lb. of a mixture of vitamins and minerals. The mixture was: manganese sulfate, 8.0g.; commercial B vitamin concentrate, 25.0 g.; B_{12} supplement (12.5 mg./lb.), 25.0 g.; choline supplement (25%), 20.0 g.; and cod liver oil (2250A-750D), 227.0 g. After a 2-wk. period on these diets the chicks were weighed individually. Group feed consumption was also determined at this time, and the gains per g. of protein consumed were calculated from these data.

The term, protein quality, as used in these experiments is defined as the ability of the protein supplement, cottonseed meal, to promote growth under established conditions.

Analytical Methods. The moisture, oil, nitrogen, crude fiber, and free and total gossypol contents of the meals were determined according to the Official Methods of the A.O.C.S. (12), available lysine was determined essentially as described by Conkerton and Frampton (13), and nitrogen solubility was measured by dispersion in 0.02 *N* NaOH as suggested by Lyman *et al.* (14). Total and reducing sugars and total phosphorous were determined as specified in the Methods of Analysis of the A.O.A.C. (15).

TABLE IV

Chick Feeding Data

Meal in diet[a]	Mean figures for three groups of chicks after two weeks' growth period			
	Feed consumed, g.	Protein consumed, g.	Weight gain, g.	Protein efficiency, g.gain/g. protein consumed[b]
M-1	1026.00	123.12	289.66	2.32
M-2	1221.33	146.56	363.33	2.48
M-3 & 6	1082.66	129.92	313.00	2.40
M-3 & 6(A)	967.66	116.12	229.66	1.98
M-4	1080.00	129.60	306.33	2.36
M-5	1177.66	141.32	319.66	2.28
M-7	1225.66	147.08	364.66	2.48
M-8	1153.00	138.36	374.33	2.71
M-9	1264.00	151.68	372.33	2.46
M-10	1090.00	130.80	313.66	2.39
M-11	1213.33	145.60	370.66	2.55
106	1104.33	132.52	290.33	2.19
107	1002.00	120.24	206.33	1.71
108	937.33	112.48	165.66	1.46
109	1070.66	128.68	255.00	1.98
110	935.00	112.20	180.66	1.61
111	984.00	118.08	224.33	1.91
112	1028.33	123.40	249.00	2.01
113	722.33	86.68	116.00	1.27
SBOM	1701.33	204.16	711.33	3.48

[a] See Tables I and II for the preparation and chemical properties of A:H:W extracted meals M-1 through M-11; the chemical properties of comparison meals 103 through 113 are given in Table III.

[b] Standard error of the mean for their determination is 0.1.

The composition of each A:H:W solvent was determined by a method due to King (16). A measured volume of the solvent was mixed with an excess of water, centrifuged in a stoppered Babcock bottle, and the volume of the supernatant layer of hexane measured. Water was determined by mixing another aliquot of the solvent with an excess of hexane in an A.S.T.M. D96 calibrated sedimentation tube; after centrifugation, the volume of the bottom layer of water was measured. By employing suitable constants and calibration curves, the percentages of hexane and of water were calculated from these data. The percentage of acetone was estimated by difference.

Results and Discussion

The data on the A:H:W extractions are given in Table I, and chemical characteristics of the A:H:W meals are presented in Table II. Table III gives the chemical characteristics of the comparison meals, and the chick feeding results for all of the meals are recorded in Table IV. To facilitate comparison of meal properties and extraction procedures, Table V was assembled.

Employing oil and gossypol contents of the meals as criteria, the trend of data in Table V indicates that the efficiency of the extraction tends to increase as the water content of the solvent increases from 0.0 to 3.0%, the effect being rather pronounced for the removal of free gossypol. A similar trend was noted when moist butanone was employed to extract cottonseed flakes (17).

When the A:H:W solvent containing 3% water was used, increasing the duration of the first solvent pass from 3 or 3.5 hr. (yielding Meals M-1 and M-5, respectively) to 17 hr. (yielding Meal M-2) led to more thorough extraction of the oil, but evidently caused some "fixation" of the gossypol. This effect might be rationalized by assuming that the longer soaking period permitted a more extensive combination between meal constituents and the gossypol which had been freshly liberated from the pigment glands. The relatively high oil and gossypol contents of Meal M-4 reveal that the efficiency of extraction was greatly reduced if the flakes were not preconditioned prior to treatment with the solvent. It was noted that the flakes which were not preconditioned tended to crumble during extraction, leading to a relatively nonporous cake of poor draining characteristics. Possibly this mechanical effect accounts for the low efficiency of extraction (18).

An extraction schedule comprising a first solvent pass of short duration (3 hr.), followed by multiple (12–13) passes, yielded a meal, M-3&6, which had the anticipated low oil and gossypol contents, together with a high available lysine content. Autoclaving a portion of this meal for 10 min. at 107–110°C. yielded a product, Meal M-3&6(A), which

TABLE V[a]

Comparison of Meal Properties and Extraction Procedure

Meal	Composition of A:H:W solvent, % (v/v)	Duration of first solv. pass, hr.	Total No. solv. passes	Oil in meal, %[b]	Gossypol in meal, %[b]		Available lysine in meal, g./16 g. N	Mean individual chick gain,[c] g.
					Total	Free		
M-7	56.7:43.3:0.0	3	6	1.05	0.58	0.268	4.30	36.5
M-8	56.1:42.9:1.0	3	6	1.00	0.71	0.313	4.32	37.4
M-11	42.6:56.2:1.2	3	6	1.15	0.67	0.229	4.02	37.1
M-9	55.5:42.5:2.0	3	6	1.02	0.62	0.164	4.36	37.2
M-2	52.7:44.3:3.0	17	6	0.30	0.49	0.054	4.11	36.3
M-1	52.6:44.4:3.0	3	6	0.58	0.37	0.032	3.98	29.0
M-5	51.6:45.4:3.0	3.5	6	0.67	0.39	0.052	3.98	32.0
M-10	55.0:42.1:2.9	3	6	0.77	0.49	0.077	3.98	31.4
M-3&6	51.7:45.3:3.0[d]	3	13–14	0.16	0.34	0.026	4.22	31.3
M-4[e]	51.6:45.4:3.0	17	6	2.70	0.62	0.065	3.86	30.6
M-3&6(A)	51.7:45.3:3.0[d]	3	13–14	0.18	0.30	0.011	3.49	23.0
SBOM	.....	.....	.....	.....	.....	.....	5.44	71.1

[a] Regrouped data from Tables I, II, and IV.
[b] Percentages on a moisture-free basis.
[c] Mean weight gain per group of chicks divided by 10, the number of chicks per group.
[d] Mean composition of solvents used for Extractions 3 and 6.
[e] The flakes used for Extraction 4 were not preconditioned; all other meals were prepared using preconditioned flakes (see text).

was decidedly low in available lysine and in nitrogen solubility (see Table II). According to earlier work (1,2), these alterations should be reflected in decreased protein quality. The low chick weight gains noted for Meal M-3&6(A), together with its decreased protein efficiency, substantiated previous observations.

As indicated in Tables II, III, and V, all of the meals examined covered a wide range of available lysine values and chick growth figures. Comparison Meal 113, the poorest meal with an available lysine content of 1.58 g./16 g. *N*, promoted a mean individual chick weight gain of only 11.6 g. Soybean oilseed meal was the best supplement, with the corresponding figures of 5.44 g./16 g. *N* available lysine and 71.1 g. growth. Figure 1 reveals that a plot of the logarithm of the mean individual chick gains against the available lysine contents is very nearly a straight line, and that the A:H:W extracted meals were superior to the commercial-type meals in growth-promoting capacities, the one noteworthy exception being Meal M-3&6(A). As described above, this meal had been autoclaved with a resultant lowering of available lysine and nitrogen solubility.

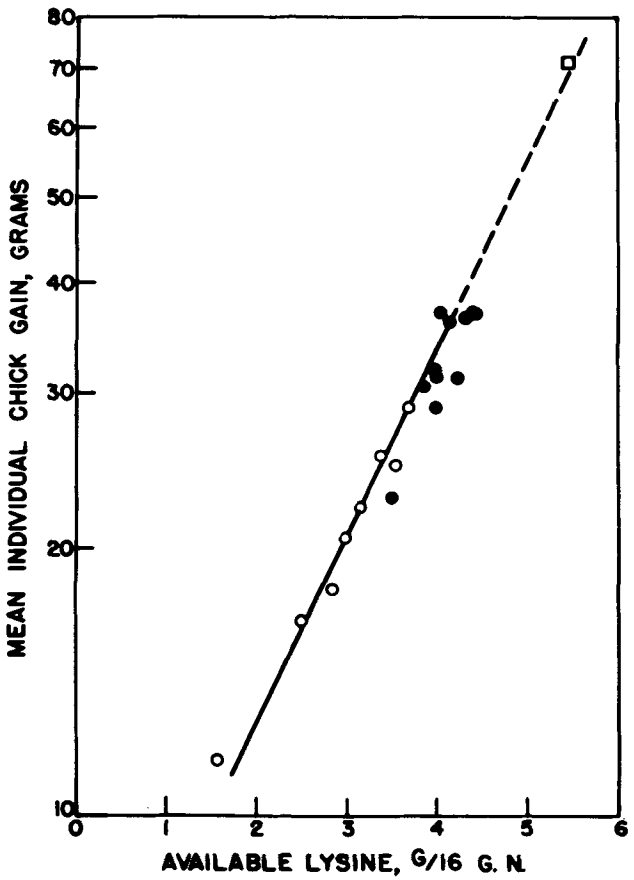

FIG. 1. Chick growth as related to available lysine contents
●, A:H:W meals
○, comparison meals
□, soybean oilseed meal.

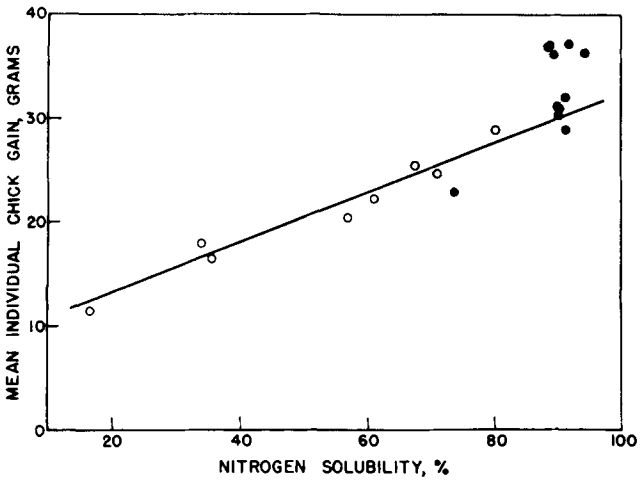

FIG. 2. Chick growth as related to nitrogen solubility (0.02 *N* sodium hydroxide)
●, A:H:W meals
○, comparison meals.

Figure 2 is a plot of the growth-promoting properties of the meals against meal nitrogen solubility (in 0.02 *N* NaOH). This latter characteristic has been proposed as a criterion of meal protein quality (14). In general, these results agree with the findings of Conly (19) in that there seems to be a fair correlation between chick growth and nitrogen solubility as long as the latter figure is 80% or lower; above this value the relationship fails.

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Hydrogenation of Linolenate. IV. Kinetics of Catalytic and Homogeneous Chemical Reduction[1]

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Kinetics for consecutive reactions of octadecatrienoate to octadecadienoate to octadecenoate have been studied with the aid of radioisotopic tracers and gas chromatography. Evidence for a triene to monoene shunt has been obtained. Similarly, the chemical reduction with hydrazine has been studied, but no evidence for this anomalous behavior was obtained. Methods to determine reaction rates from these kinetic measurements are discussed.

BAILEY summarized in 1949 the kinetic information available on catalytic reduction of triglyceride oils containing linolenic acid (1). First order equations for estimating the relative reaction rates of oleic, isolinoleic, linoleic, and linolenic acids were adapted by him. Under "nonselective" conditions, the ratio of reaction rates of linolenate to linoleate of 1.7 was observed, whereas under "selective" conditions a ratio of 2.5 was found. Consideration of reaction rates led him to conclude that a large portion of linolenate was directly reduced to oleate not stopping at the linoleate stage. No further publications on the rates of hydrogenation of linolenate have appeared since Bailey's summary.

In this work C^{14}-labeled fatty acid methyl esters were used to study the kinetics of catalytic reduction. Newly developed procedures for monitoring gas chromatography for labeled compounds (2) were exploited to estimate the specific activity of individual esters, to measure the rates of hydrogenation of linolenate and linoleate and to study the steps of conversion of linolenate to octadecenoate, i.e., the "oleate shunt." These kinetics for heterogeneous catalysis were compared with those for the homogeneous chemical reduction of linolenate by hydrazine. This single-phase chemical reaction, in contrast to heterogeneous catalysis, is characterized by either minimal, or no shift, in position (3) or geometric configuration of double bonds (4). Also, in contrast to catalytic hydrogenation and its varying order of reaction, first order kinetics are observed for chemical reduction; no evidence is apparent for the oleate shunt in homogeneous phase reduction. The reaction rates for the chemical reduction of octadecatrienoate, octadecadienoate and octadecenoate appear in approximately the same ratio as the number of double bonds present.

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Experimental

An equimixture of methyl linolenate and methyl linoleate by weight was hydrogenated in all the experiments described. This procedure permitted relative reaction rates for the two esters to be determined under identical conditions in each experiment. It minimized variations caused from run to run by uncontrolled differences in conditions such as catalyst concentration or activity, pressure, stirring, and inhibitors.

Catalytic hydrogenations presented are of two types: a) those in which C^{14}-labeled linolenate is added to the equimixture and b) those in which C^{14}-labeled linoleate is added.

Methyl linolenate and methyl linoleate were isolated in gas chromatographically pure state by the preparative countercurrent distribution technique (5). Linolenate randomly labeled with C^{14} was isolated by countercurrent distribution from soybeans grown in an atmosphere containing $C^{14}O_2$ (6). Linoleate, carboxy labeled with C^{14}, was obtained from Nuclear-Chicago Corp.

Catalytic hydrogenation experiments were carried out on a 0.6-g. scale with approximately 3 microcuries of added labeled ester. A 50-ml. flask with slightly rounded bottom and a magnetic stirrer comprised the reactor. A temperature of 140°C., hydrogen gas at atmospheric pressure, and 0.5% of a commercial catalyst (electrolytically reduced nickel on kieselguhr) were used. The uptake of hydrogen was followed manometrically. Samples were removed through a rubber serum cap seal with a hypodermic needle and syringe at appropriate intervals of hydrogen absorption. At each sampling, approximately 50 mg. were removed, weighed in the syringe and made to 1 ml. in pentane-hexane solvent. After the catalyst settled out, 20 μl. of solution containing approximately 1 μl. of esters was injected into a 7-ft. gas chromatographic column packed with 20% (w/w) of polyethylene glycol succinate on 80- to 100-mesh Chromosorb. A conventional gas chromatograph with thermal conductivity detector was used. Eluent solutes were