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On the acceleration of lipase activity caused by the action of substances containing vitamin A

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ON THE ACCELERATION OF LIPASE ACTIVITY CAUSED BY THE ACTION OF SUBSTANCES CONTAINING VITAMIN A

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ON THE ACCELERATION OF LIPASE ACTIVITY CAUSED BY THE ACTION OF SUBSTANCES CONTAINING VITAMIN A

I. Introduction.

The discovery of the presence of certain growth promoting factors and malnutrition correctives in foods is yet so recent that their isolation and nutritional functions are not known at the present time. Something is known, however, of the properties of the more outstanding of these factors, termed originally by C. Funk, Vitamines, and changed later by J. C. Drummond to Vitamins in order to avoid any implication as to chemical constitution. Thus, Vitamin A[1] has been found to be associated with fats and oils which have their origin in animal life. In plants, however, Vitamin A is not generally associated with the fats as it is not readily extracted from such sources by means of fat solvents. Vitamin A, it has been found, is not destroyed to any great extent by a saponification process involving the use of a non-aqueous caustic alkali. It is, however, destroyed slowly by heat and much more rapidly if oxygen is present during the heating. Thus it is noted that aerating butter fat at 100° C. for four hours inactivates the vitamin. Vitamin A, in some unknown manner, promotes the growth of animals when present in amounts almost unbelievably small. It prevents and cures zerophthalmer if this disease of the eye has not been permitted to go too far. Vitamin A is distinguished from the other vitamins, B and C,

by its solubilities and physiological effects. As to its nutritional function, it may be stated that Vitamin A appears to be associated with fat metabolism as well as with the other metabolic process requisite for growth and maintenance. The exact manner in which it functions during fat metabolism does not appear to be known. J. C. Drummond[2], who has worked on this, was not able to obtain any direct relationship between Vitamin A and the synthesis of fats from free fatty acids when fed to rats lacking this vitamin. It appears from his work that the power of absorbing fatty acids is retained after the rats have begun to show the characteristic symptoms of lack of A. Later work by Osborne and Mendel[3] indicates that fats are dispensable constituents of the diet provided there is present a suitable source of Vitamin A.

The various enzymes contained within living tissues as in the digestive fluids of animals are indisputably closely connected with the metabolic processes taking place within the animal body. Since any change in the velocity of the enzyme reactions is bound to affect the rate of synthesis and decomposition of vital tissue, it should not be surprising if one function of the vitamin is to bring about the normal velocity of enzyme reactions. In this connection it is worthy to remark that as yet no enzyme reaction has been known to proceed as fast in vitro as in vivo. It may be that part

of this difference is due to the absence of vitamins in enzyme reactions carried out in vitro.

Few investigations of the effect of vitamins upon enzymes have been carried out. Harden[4] calls attention to an unaccountably increased activity of yeast juice which contains zymase, if again boiled yeast juice is added. In the same publication[5] he refers to Abderholden and Schauman's work, calling attention to the fact that addition of various fractions of an acid extract of yeast to yeast juice causes greater activity on the part of the zymase. It is entirely probable that Bios, the yeast growth stimulant, is associated with this unusual acceleration, inasmuch as the conditions of experimentation were such that Bios could be safely assumed to be present. If this was to be found true, and some very preliminary work on the part of the author seems to indicate that this is so, it can be inferred by analogy that the vitamins may also be concerned as acceleration of certain of the enzymes of the animal body.

M. J. Villaroel[6] has observed that extracts of Vitamin B, obtained from yeast, pancreas and from testes augment the action of catalose extract of liver. These extracts were also found to accelerate castor bean lipase and the amylolytic action of laka diastase and of amylose. He suggests that the vitamins function as asteration of enzymes in vivo. However, the criticism of A. Sordelle[7] that the extracts

containing B are complex and that it is necessary to show that the stimulation is due to Vitamin B is justified. U. Sanimorteno[8], working with vitamin extracts, found that zymase activity was accelerated, whereas the same vitamin extract had little action upon pepsin and a small positive action upon trypsin and diastase. However, the acceleration in the case of trypsin and diastase was very small as compared to the acceleration obtained with zymase. Catalase activity was decreased or increased considerably depending upon the reaction of the vitamin extract. His work would indicate that the vitamins exert a specific action upon enzymes. It is unfortunate that in his publication he does not specify what vitamin he worked with nor the mode of preparation. Tanaka Yoshio[9] made the observation during the course of his work on the castor bean lipase that a water extract of lipase accelerated lipase activity. There is found, therefore, in the literature record of a few instances in which enzyme activity has been accelerated by extracts made from sources now known to contain vitamins. However, it must be emphasized that in none of the cases noted have the investigations been carried to such a stage that factors other than vitamins were eliminated.

The present problem grew, therefore, out of a realization of the lack of inadequate explanation of the increase in enzyme action by certain extracts and also out of a likelihood that Vitamin A, due to its presence in the fat vehicle, must come into contact with the enzyme lipase.

II. Statement of the Problem.

The object of the work presented by the author is to determine:

1. The effect of Vitamin A concentrates upon lipase action in vitro.
2. To eliminate as far as possible factors other than Vitamin A to which acceleration if noted might be ascribed.
3. The effect of Vitamin A is found in the common Vitamin A vehicles upon lipase action in vitro.
4. To establish if possible a parallelism between animal growth induced by Vitamin A and accelerated lipase activity in vitro caused by presence of Vitamin A concentrates.

III. Materials and Methods.

The most suitable and convenient method for the estimation of lipase activity appeared, after some preliminary work, to be the hydrolysis of ethyl butyrate by the lipase contained in commercial preparation of pancreatin with subsequent determination of the butyric acid by standard alkali after a Reichert-Meissl distillation. This method gives quantitative measurements with a fair degree of accuracy, provided the operator is careful to observe certain precautions at various stages of the analysis. The hydrolysis of ethyl butyrate is a familiar reaction and has been extensively studied from various points of view. A consideration of these studies is not within the scope of this thesis and the author wishes to call attention only to certain phases that were met with in the course of the work.

A. Lipase:

The commercial preparation of pancreatin is a glycerol extract of the pancreas of a sheep or of a pig. Altho much of the fat adhering to the pancreas is removed before extraction, there is no doubt considerable opportunity for glycerol to dissolve fatty material along with the enzymes. It was noticed during the preliminary work that small amounts of a substance volatile with steam but insoluble in water was obtained in the distillate receiving flasks. Presumable this substance was composed of volatile insoluble fatty acids. To

avoid having any fatty acids other than the butyric acid derived from ethyl butyrate present it was deemed advisable to extract the pancreatin by means of ether for a period of 12 hours. It was found that the ether had extracted considerable fat from the pancreatin. The extracted pancreatin was about as active as the unextracted.

P. B. Hawk[10] states that pancreatic lipase is very unstable and is rendered inert by the presence of acid. To avoid this inhibiting action by hydrogen ions it was decided to use the slightly alkaline buffer, Na_2HPO_4, in the medium.

Preliminary experiments indicated that 0.5 gms. pancreatin was a convenient quantity to cause measurable hydrolysis in the course of four to ten hours. 0.5 gms. of pancreatin were therefore taken up in 5 cc. of the Na_2HPO_4 medium, in which the pancreatin readily dissolved, forming an almost transparent light brown solution.

B. Ethyl Butyrate:

Ethyl butyrate when taken from stock was found to be slightly hydrolyzed. It is of decided advantage from an analytical point of view that the ethyl butyrate be as free as possible from butyric acid. Stock ethyl butyrate that proves to be very acid may readily be purified by washing the ethyl butyrate with dilute alkali a few times and then removing the alkali by washing with distilled water. The ethyl butyrate may then be freed from the greater part of the water by means

of a separatory funnel and the last traces removed by means of anhydrous $CaCl_2$. The pure ethyl butyrate is then obtained by distillation.

C. Water:

The water used in carrying out the experimental was ordinary distilled water boiled and cooled without shaking immediately before conducting the Reichert-Meissl distillation. This is an essential procedure inasmuch as phenolphthalein, the indicator used during the titration with standard alkali, is quite sensitive to carbon dioxide.

D. Vitamin A Concentrates:

The Vitamin A concentrates were made according to Steinbock, Sell, and Buell[11]. 300 grams of butter fat were saponified with 600 cc. of a 20% alcoholic potassium hydroxide. The saponification was allowed to proceed at room temperature for a period of four hours. The soaps were then dissolved in 2500 cc. of water. The alcohol water soap solution thus formed was extracted three times with diethyl ether. The ether extracts were combined and washed repeatedly with large volumes of water to remove soaps. Five to seven washings usually sufficed to remove all soap from the ether extract. The extract was then transferred to a distilling flask and the greater part of the ether distilled off. After the concentrated extract had been transferred to a small crystallizing dish the remaining ether was allowed to evaporate at room

temperature. It was found that from 300 grams of the original fat about 3 grams of concentrate or unsaponifiable matter was obtained.

E. Production of Film of Vitamin A Concentrate:

Inasmuch as Vitamin A is contained in the unsaponifiable matter from the fat, it seemed wise to produce if possible a film of the unsaponifiable matter in order to insure as far as possible intimate contact between the enzyme lipase and the vitamin. It is also essential that the film vehicle should be as inert as possible. A fine grade of sea sand offered a solution to this difficulty and was tried with success. The film was produced by dissolving a small quantity of Vitamin A concentrate in ether and evaporating the ether after transferring to a weighed quantity of specially treated sea sand. To induce as far as possible a film of uniform thickness, the sand was stirred with a glass rod during the last stages of evaporation.

The special treatment referred to in connection with the sea sand consisted in incineration at red heat for two or three hours, treatment with hot aqua regea, and washing with distilled water until a negative chloride test was obtained with silver nitrate. The sand was then filtered off, put aside in a dust-free compartment to dry.

The amount of the Vitamin A concentrate deposited upon sea sand was in the preliminary experiments approximately 2% by weight of the final product. In the later, more carefully

controlled experiment, the Vitamin A concentrate sand mixture contained very nearly 25% by weight.

F. Production of Film of Various Fats:

Films of fats and oils from various sources were deposited upon sea sand in a manner similar to the one used for the production of film of Vitamin A. These films were used in connection with the studies on the effect of Vitamin A as found in the common Vitamin A vehicles upon lipase action in vitro.

G. Nomenclature:

The product formed by the deposition of a film of Vitamin A concentrate upon sand is called, for convenience, Vitamin A concentrate (B.F.) sand preparation, if the concentrate is obtained from butter fat.

The similar product made from the fats or oils is called, fat or oil sand preparation. For example, the product obtained by deposition of a film of cod liver oil on sand would be known as the C.L. oil sand preparation.

H. Preservative:

Toluol was used as a preservative. For any series of determinations the amount was kept the same. In general four drops were added to each reaction flask.

I. The Reichert-Meissl Distillation:

The butyric acid formed by lipase hydrolysis was liber-

ated by the addition of 5 cc. of dilute H_2SO_4 (5 cc. = 1 gm. H_2SO_4). The contents of the digestion flask were then transferred to a 500 cc. balloon flask which served as a distilling flask. The washings from the digestive flask were also transferred quantitatively. About 50 cc. of CO_2 free distilled water was used for effecting this transfer. 250 cc. of CO_2 free distilled water was then added and a piece of porous plate dropped in. It was found that in the controls forming took place during distillation and this was remedied by the addition of a drop or two of cottonseed oil. The connection between the balloon flask and the condenser was made with a Kjeldahl nitrogen bulb. All glassware was rinsed in warm CO_2 free distilled water immediately before making a distillation. The butyric acid was distilled and caught in 500 cc Erlenmeyer flasks. 250 cc. of distillate proved an efficient volume to distil. The distillation was carefully controlled in all experiments, usually requiring from two to three hours for each series.

One of the difficulties met with during the distillation was bumping. This was violent in some cases, due to the presence of the sea sand. When the transfer of the contents of the lipase reaction flask was made by such a means as to avoid transfer of sand, the bumping was almost entirely overcome. The danger in bumping consisted in the possibility of H_2SO_4 being carried over into the distillate.

J. Titration of Butyric Acid:

N/10 NaOH was added to the distillate receiving flasks until a decided pink coloration remained for more than one minute. Phenolphthalein was used as the indicator.

IV. Experimental.

A. The Effect of Vitamin A Concentrate from Butter Fat upon Lipase Activity. Ethyl Butyrate as Substrate.

TABLE I

Exp. No.	Flask No.	Reaction Constituents: Gms. Pancreatin	Gms.* Vitamin A. (B.F.) Sand Prep.	Gms. Na_2HPO_4 12 H_2O	Cc. H_2O CO_2 free	Cc. Ethyl Butyrate	Tolnol Drops	Hrs. Incubation	Temp.	Cc. of N/10 NaOH to neut. Butyric Acid
19	0	0.500	0.000	0.500	5.00	1.00	Approx. 0.25 c.c. Not Measured	4½	25°C.	6.29
	1	"	0.500	"	"	"		"	"	8.44
	2	"	1.000	"	"	"		"	"	9.03
	3	"	1.500	"	"	"		"	"	10.39
28	0	@ "	0.00	#3.00cc.	2.00	"	9	4½	30°C.	5.67
	1	"	0.500	"	"	"	"	"	"	6.12
	2	"	1.000	"	"	"	"	"	"	6.51
	3	"	1.500	"	"	"	"	"	"	6.79
	0	@ "	0.000			0.40	"		"	5.10
	1	"	1.500			"	"		"	7.69
	2	"	1.000			"	"		"	8.22
	3	"	1.500			"	"		"	8.93

*Enuf Vitamin A concentrate added to sea sand to inhibit the natural fluidity of the sand. Approximately 2% by weight.

@Pancreatin, ether extracted for 12 hours.

#Solution of Na_2HPO_4 12 H_2O. 1 cc. = 0.25 gms. Na_2HPO_4 12 H_2O.

The acceleration noted in terms of cc. N/10 NaOH has been plotted in Figs. 1, 2 and 3. The upward trend of the curve is decisive, showing to greater advantage in Figs. 1 and 3 than in Fig. 2.

FIG. 1

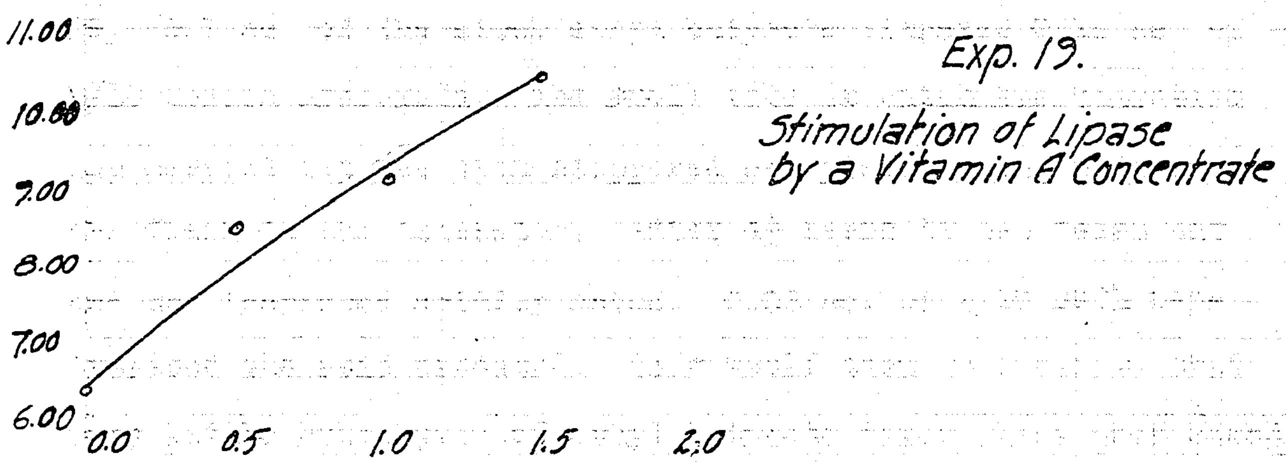


FIG. 2.

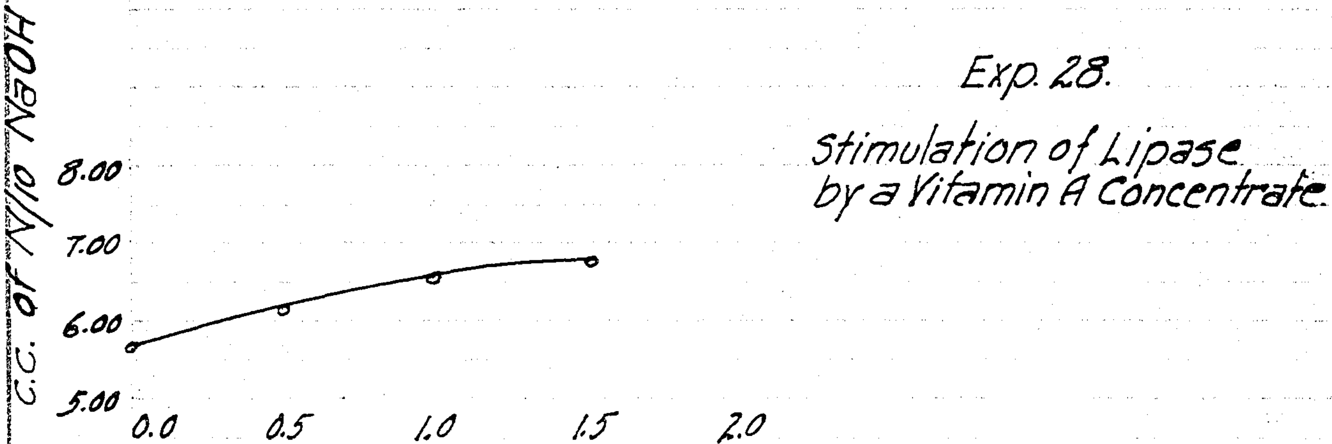
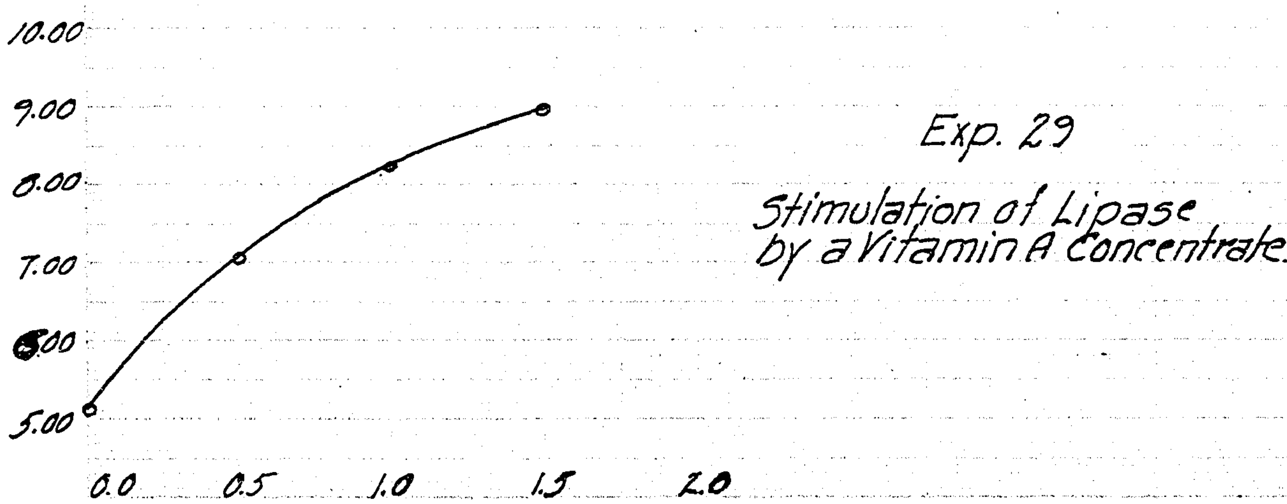


FIG. 3.



Gms. Vitamin A (B.F.) Sand Preparation

The acidity of the ethyl butyrate was determined in Exp. 29. 0.1 cc. of the stock ethyl butyrate titrated 0.15 cc. of N/10 sodium hydroxide. The small tube in which the titration was carried out was then stoppered and placed along side of the flask in the incubator. After 4½ hours it was taken out and the increased acidity noted. 0.02 cc. of N/10 NaOH neutralized the acid produced. This would seem to indicate that very little hydrolysis of ethyl butyrate takes place when acted upon by water containing sodium butyrate during the period of incubation.

B. The Effect of Factors Other than Vitamin A Concentrates upon Lipase Activity.

1. Film of Cottonseed Oil on the Surface of Sand.

TABLE II

Exp. No.	Flask No.	Reaction Constituents: Gms. Pancreatin in E.E.	Gms. C.S. Oil Sand Prep.*	Cc. Na_2HPO_4 Sol.	Cc. H_2O (CO_2 free)	Cc. Ethyl Butyrate	Tol-uol Drops	Hrs. Incubation	Temp.	Cc. of N/10 NaOH to neut. Butyric Acid
34	0	0.500	0.000	3.00	5.00	0.50	6	8¾	30°C.	7.49
	1	"	0.20	"	"	"	"	"	"	8.40
	2	"	0.40	"	"	"	"	"	"	8.79
	3	"	0.60	"	"	"	"	"	"	8.55
39	0	0.500	0.000#	2.00	5.00	0.50	5	5 2/3	30°C.	8.48
	1	"	0.150	"	"	"	"	"	"	8.10
	2	"	0.300	"	"	"	"	"	"	7.77
	3	"	0.450	"	"	"	"	"	"	7.45
	4	"	0.600	"	"	"	"	"	"	8.17
	5	"	0.750	"	"	"	"	"	"	7.88
	6	"	0.900	"	"	"	"	"	"	8.40
	7	"	1.050	"	"	"	"	"	"	7.70
	8	"	1.200	"	"	"	"	"	"	7.82
	9	"	0.000	"	"	"	"	"	"	6.75

*0.0621 gms. of cottonseed oil dissolved in ether. 2 gms. of specially treated sea sand introduced and ether allowed to evaporate. Stirred during last stages of evaporation.

#0.2080 gms. cottonseed oil on 7.0558 gms. of sea sand (specially treated).

It was thought that perhaps one of the factors that might be the cause of the acceleration noted in experiments 19, 28, and 29 was the presence of a fat like film on the sane. Experiment 34 was

therefore made. The results of this series indicates a slight acceleration which could not, however, be reproduced in experiment 39. It is thought therefore that the effect of a film of oil upon sand is not the cause of the acceleration noted. Cottonseed oil was selected to produce the film as the oil has been found to be deficient in Vitamin A.

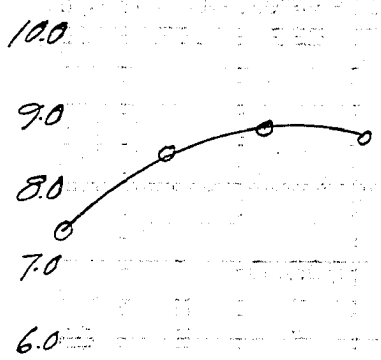
2. The Effect of Specially Treated Sea Sand on Lipase Activity.

TABLE III

Exp. No.	Flask No.	Reaction Constituents: Gms. Pancreatin in E.E.	Gms. Sea Sand	Cc. Na_2HPO_4 Sol.	Cc. H_2O (CO_2 free)	Cc. Ethyl Butyrate	Toluol Drops	Hrs. Incubation	Temp.	Cc. N/10 NaOH to neut. Butyric Acid
26	0	0.500	0.00	3.00	2.00	1.00	5	4½	30°C.	6.22
	1	"	0.50	"	"	"	"	"	"	6.18
	2	"	1.00	"	"	"	"	"	"	4.80
	3	"	1.50	"	"	"	"	"	"	5.04
26a	0	"	0.00	"	"	"	"	"	"	9.32
	1	"	0.50	"	"	"	"	"	"	9.65
	2	"	1.00	"	"	"	"	"	"	--
	3	"	1.50	"	"	"	"	"	"	9.60

It is concluded from this data that the increasing amounts of sea sand used as the carrier for the Vitamin A concentrate is not the source of the stimulation noted.

FIG. 4

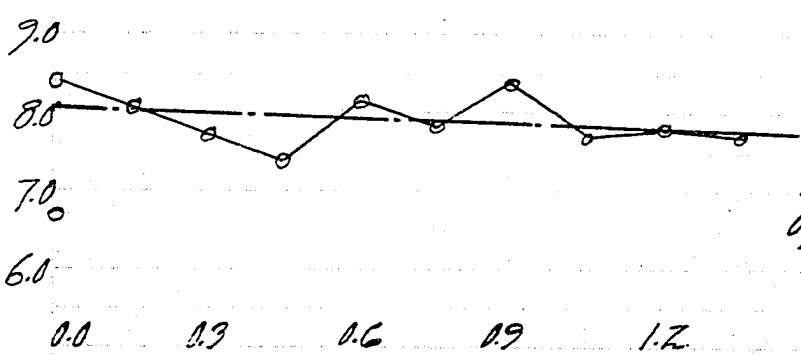


c.c. of N/10 NaOH

Exp. 34

The Effect of a Film of Oil on Lipase Activity

FIG. 5



Grams Sand Oil Preparation.

Exp. 39.

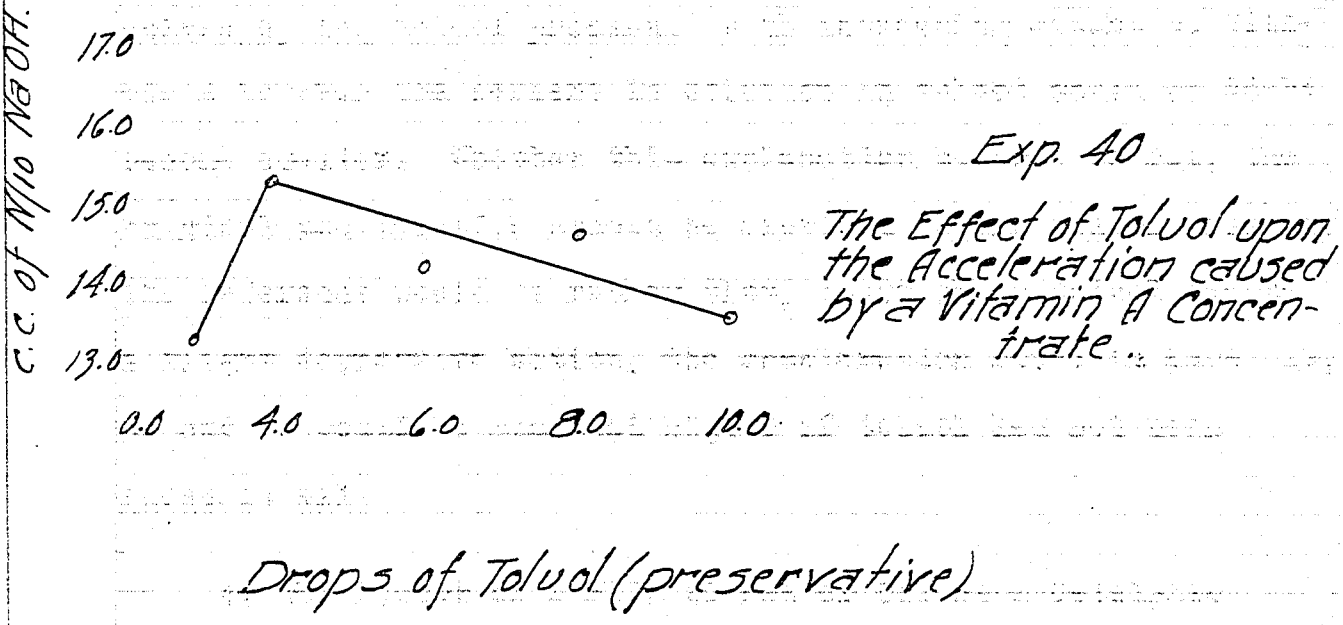
The Effect of a Film of Oil on Lipase Activity.

3. The Effect of Toluol on Lipase Activity in the Presence of Vitamin A Concentrate.

TABLE IV

Exp. No.	Flask No.	Reaction Constituents: Pancreatin	Vit. A (B.F.) Sand Prep.	Cc. Na_2HPO_4 Sol.	H_2O (CO_2 free)	Cc. Ethyl Butyrate	Toluol Drops	Hrs. Incubation	Temp.	Cc. N/10 NaOH to neut. Butyric Acid
40	1	0.500	0.000	3.00	2.00	0.50	2	$8\frac{3}{4}$	30°C.	13.22
	2	"	0.300	"	"	"	4	"	"	15.28
	3	"	"	"	"	"	6	"	"	14.14
	4	"	"	"	"	"	8	"	"	14.68
	5	"	"	"	"	"	10	"	"	13.52

FIG. 6



Exp. 40

The Effect of Toluol upon the Acceleration caused by a Vitamin A Concentrate.

The results presented in this table show that varying the toluol as indicated tends to reduce the acceleration due to Vitamin A concentrate. The cause of this decrease was not investigated. It is, however, possible that the increased amount of toluol has some solvent action upon the Vitamin A concentrate, thereby withdrawing the Vitamin A from the seat of hydrolytic action. Altho the amount of toluol in Exp. 28 and 29 was kept constant throughout the series, it does not necessarily follow that the quantitative action of toluol was the same in each flask, if its action is explained on a solvent basis. Flask No. 2 contained very little of the Vitamin A concentrate and a large percent of this may have been dissolved by the toluol present. With increasing amount of Vitamin A however the percent in solution in toluol would no doubt become smaller. Whether this explanation be true or not, the original acceleration cannot be ascribed to the toluol present. The inference would be rather that, inasmuch as toluol exerts a slight depressive action, the acceleration noted in Exps. 19, 28 and 29 would be somewhat higher if toluol had not been added at all.

4. The Effect of a Film of Fat or Oil as a Catalytic Agent in the Hydrolysis of Ethyl Butyrate.

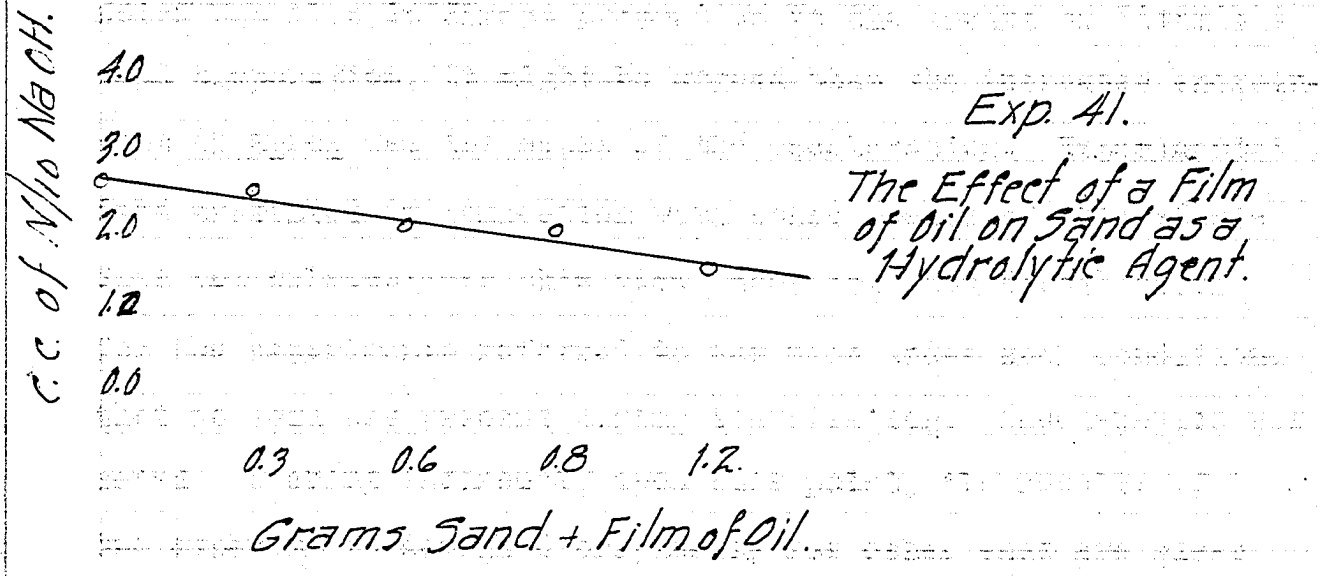
C. C. Warden[12] concluded from his work as to the nature of alcoholic fermentation, that the fermentation is due to a catalytic process operating at the surface of the yeast cells,

at the colloidal surface of yeast juice (zymase), and at artificial surfaces composed of specific fat complexes similar to those found to be present in yeast cells, and that the enzyme of yeast may be regarded as belonging to the cellular antigens." He based these conclusions upon the fermentation of certain sugars by fibrin carrying an appropriate film of Na oleate. The experimental conditions used by Warden are in some respects similar to those used in the experiments presented in this thesis. For this reason, an experiment was outlined to determine if a film of fat or oil will act under the conditions used as a hydrolytic agent for ethyl butyrate. The results are presented in Table No. V. Evidently the hydrolysis is not accelerated. The rather high titer is due in part to some butyric acid performed by hydrolysis. Some butyric acid has no doubt been formed thru action of the medium used.

TABLE V

Exp. No.	Flask No.	Reaction Constituents: Pancreatin	Oil (CS) Sand Prep.	Cc. Na_2HPO_4 Sol.	Cc. H_2O CO_2 free	Cc. Ethyl Butyrate	Toluol Drops	Hrs. Incubation	Temp.	Cc. of N/10 NaOH to neut. Acid
41	0	0.000	0.000	3.00	2.00	0.50cc.	4	8½	30°C.	2.72
	1	"	0.300	"	"	"	"	"	"	2.65
	2	"	0.600	"	"	"	"	"	"	2.20
	3	"	0.900	"	"	"	"	"	"	2.15
	4	"	1.200	"	"	"	"	"	"	1.58

FIG. 7.



Exp. 41.

The Effect of a Film of Oil on Sand as a Hydrolytic Agent.

5. The Effect of Sand during the Distillation of the Butyric Acid.

The presence of sea sand in the distillation flask caused considerable bumping. This when severe no doubt was the cause of entrainment of sulphuric acid and consequently a high sodium hydroxide titer. That unusually high values sometimes occurred is a fact. During a distillation one could readily observe that the violence of bumping increased with the increase in Vitamin A sand preparation. Since the acceleration noted was also in direct proportion to the amount of Vitamin A sand preparation, it might be argued that the increased entrainment of H_2SO_4 was the cause of the acceleration. Experimental data presented in connection with other phases of this thesis does not substantiate this view, however. The distillation for the experiments referred to was made under such conditions that no sand was present during distillation. Acceleration was noted. Bearing indirectly upon this point, the results of two experiments designed originally for other ends are given in tabular form. Experiment 31 was designed to determine if the Vitamin A concentrate accelerated lipase when that enzyme is acting in a synthetic manner. Experiment 27 was designed to determine the amount of volatile fatty acids in varying quantities of Vitamin A concentrate. In both experiments the procedure was identical in respect to the addition of sulphuric acid to liberate the butyric acid.

TABLE VI

Exp. No.	Flask No.	Reaction Constituents: Gms. Pancreatin	Gms. Vit. A BF. Sand Prep.	Cc. Na_2HPO_4 Sol.	Cc. H_2O CO_2 free	Cc. Ethyl Butyrate	Toluol Drops	Hrs. Incubation	Temp.	Cc. of N/10 NaOH to Neut. Butyric Acid
31	0	0.500	0.000	3.00	1 cc. dilute butyric acid		9	10	30°C	6.90
	1	"	0.500	"			"	"	"	7.11
	2	"	1.000	"	1 cc dilute ethyl alcohol		"	"	"	6.96
	3	"	1.500	"			"	"	"	6.70
27		"	0.000	*2.50	2.50	--	9	4	"	0.20
		"	0.500	"	"	--	"	"	"	0.21
		"	1.000	"	"	--	"	"	"	0.29
		"	1.500	"	"	--	"	"	"	0.21

*To each flask was added 0.5 gms. of Na_2HPO_4 12 H_2O in addition to the 2.50 cc. of the solution.

The results shown in Table VI indicate that entrainment of H_2SO_4 is not a factor altho sand is present in the distilling flask. It has been the experience of the author that when H_2SO_4 is carried over it is usually in such amounts that the titer is very high.

6. Determination of the Efficiency of the Distillation of the Butyric Acid.

Exp. 25. Into 4 500 cc. round bottom flasks was introduced 1 cc. of a dilute butyric acid solution. 150 cc. of CO_2 free distilled water was added and the flasks connected with the upright condensers. 125 cc. of distillate was then caught

and titrated with N 0.2117 sodium hydroxide. The results are given in the following table:

TABLE VII

Flask	Cc. of dil. Butyric Acid	Observed Cc. of NaOH N 0.2117	Actual
1	1.00	2.95	3.04
2	1.00	2.80	2.99
3	1.00	2.95	2.95
4	1.00	3.13	----

7. The Effect of Cholesterol upon Lipase Action.

Cholesterol and lecithin are common constituents of fats, especially those of animal origin. E. F. Terraine[13] has shown that lecithin increases the activity of pancreatic lipase only when present in high concentrations and then only very slightly. He used mono-butyrin as substrate. It would therefore be unusual to expect lecithin to have great stimulatory power upon lipase when acting upon ethyl butyrate as substrate.

The following experiment shows that the stimulatory power of cholesterol is extremely small probably lying within experimental error.

TABLE VIII

Exp. No.	Flask No.	Reaction Constituents: Gms. Pancreatin	Gms. Cholesterol Sand Prep.	Cc. Na_2HPO_4 Sol.	Cc. H_2O CO_2 free	Cc. Ethyl Butyrate	Toluol Drops	Hrs. Incubation	Temp.	Cc. of N/10 NaOH to neut. Butyric Acid
	1	0.500	0.000	3.00	2.00	0.50	4	4½	30°C	3.90
	2	"	0.500	"	"	"	4	"	"	4.70
58	3	"	1.000	"	"	"	4	"	"	4.98
	4	"	1.500	"	"	"	4	"	"	4.30
	5	"	2.000	"	"	"	4	"	"	4.60

8. The Effect of Sodium Oleate upon Lipase Activity.

Commercial oleic acid was treated with sodium hydroxide in such a manner that not all the oleic acid was neutralized. The resulting soap solution was allowed to dry after which it was extracted several times with ether to remove the excess oleic acid. A small shaving of sodium oleate was then ground up with specially treated sea sand. The following table shows the stimulation obtained. It will be observed in Fig. I that the values are not regular but the upward tendency is apparent.

FIG. 8

Exp. 58.

The Effect of Cholesterol upon Lipase Activity.

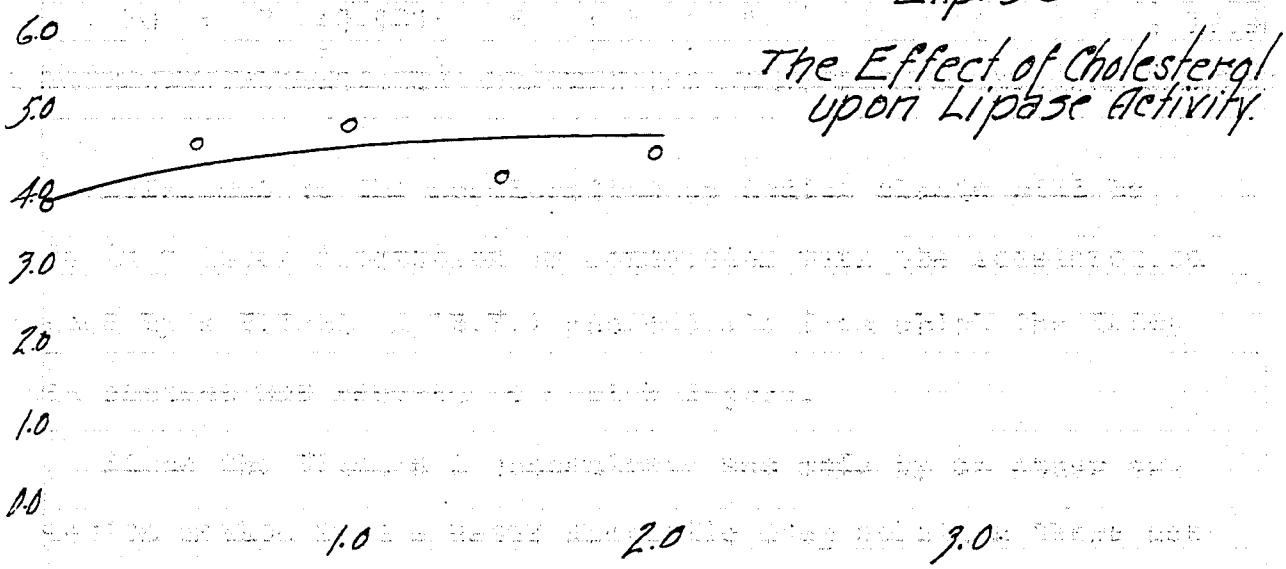


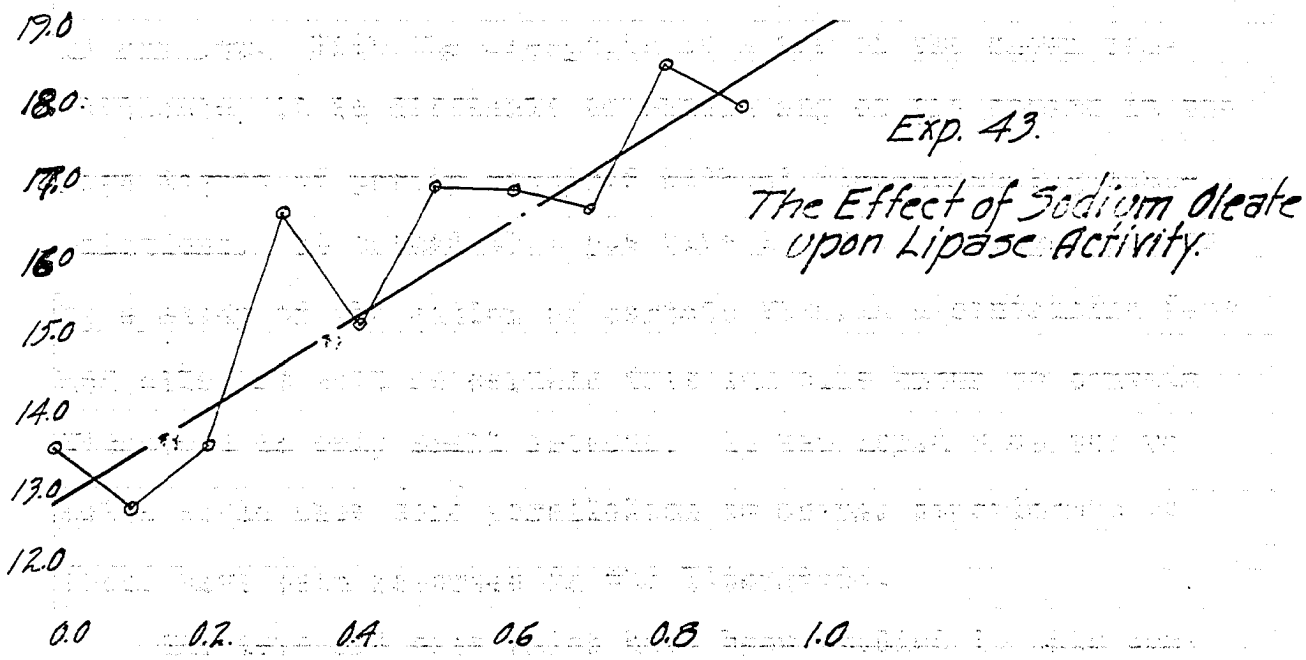
TABLE IX

Exp. No.	Flask No.	Reaction Constituents: Gms. Pancreatin	Gms. Na Oleate Sand Prep.	Cc. Na_2HPO_4 Sol.	Cc. H_2O CO_2 free	Cc. Ethyl Butyrate	Toluol Drops	Hrs. Incubation	Temp.	Cc. of N/10 NaOH to neut. Butyric Acid
	1	0.500	0.000	3.00	2.00	0.50	4	9	30°C.	13.51
	2	"	0.100	"	"	"	"	"	"	12.77
	3	"	0.200	"	"	"	"	"	"	13.57
	4	"	0.300	"	"	"	"	"	"	16.55
43	5	"	0.400	"	"	"	"	"	"	15.10
	6	"	0.500	"	"	"	"	"	"	16.90
	7	"	0.600	"	"	"	"	"	"	16.89
	8	"	0.700	"	"	"	"	"	"	16.61
	9	"	0.800	"	"	"	"	"	"	18.41
	10	"	0.900	"	"	"	"	"	"	17.90

Reference to the acceleration by sodium oleate will be made in a later discussion in connection with the acceleration caused by a Vitamin A (B.F.) concentrate from which the fatty acid content was removed to a high degree.

Since the Vitamin A concentrate was made by an ether extraction method from a water alcoholic soap solution there was no doubt ample opportunity for potassium salts of various fatty acids including oleic acid to be present in the ether extract before this was washed with distilled water. The final wash water gave no precipitate or cloudiness when tested with $AgNO_3$. The final concentrate was also tested for the presence of alkali by igniting a small amount in a weighed crucible. There could be detected no ash. When recently boiled, cooled dis-

FIG. 10



Exp. 43.

The Effect of Sodium Oleate upon Lipase Activity.

tilled water and a drop of phenolphthalein were added to the crucible, no pink coloration was detected. It is safe, therefore, to assume that potassium soaps were absent from the concentrate. The presence of fatty acids in the Vitamin A concentrate is however not to be precluded on the basis of the simple tests mentioned and will be discussed to better advantage in another connection.

C. The Effect of Vitamin A as found in the Common Vitamin A Vehicles upon Lipase Activity in Vitro.

The unsaponifiable matter obtained from fats and oils is complex. With the exception of a few of the known constituents, it is difficult to obtain any of the others in the high degree of purity required without time-consuming manipulations. It seemed that positive information might accrue by a study of the action of certain Vitamin A containing fats and oils, as well as certain fats and oils known to contain Vitamin A in only small amounts. It was hoped that the results would show some parallelism to animal experiments as these have been reported in the literature.

The fats and oils which have been studied in this connection are:

1. Vitamin A containing.
    a. Butter fat.
    b. Cod liver oil.
    c. Palm oil.

2. Not containing Vitamin A in large amounts.

a. Cottonseed oil.

b. Wheat embryo oil.

c. Nujol (paraffin oil).

d. Glycerol (included in this group for convenience).

e. Olive oil.

The data obtained for these experiments is presented in Table No. X and plotted in Figure 11-16, inclusive.

TABLE X*

Exp. No.	Fat or Oil	Flask No.	Gms. Fat or Oil Sand Prep.	Toluol Drops	Hrs. Incubation	Cc. of N/10 NaOH Required to Neut. Butyric Acid
49	Butter	1	0.000	4	6 5/6	8.89
		2	1.000	"	"	7.74
		3	0.450	"	"	8.69
		4	0.900	"	"	8.51
		5	1.350	"	"	8.84
		6	1.800	"	"	7.98
		7	2.150	"	"	8.69
51	Cod Liver Oil	1	0.000	"	4½	4.20
		2	0.450	"	"	4.69
		3	0.900	"	"	4.75
		4	1.350	"	"	4.97
		5	1.800	"	"	5.02
		6	0.000	"	"	#0.25
		7	0.450	"	"	0.29
		8	0.900	"	"	0.24
		9	1.350	"	"	----
		10	1.800	"	"	0.33
66	Palm Oil	1	0.000	2	6	4.00
		2	0.500	"	"	5.85
		3	1.000	"	"	4.88
		4	1.500	"	"	6.14
		5	2.000	"	"	5.73
		6	2.500	"	"	5.35
		7	3.000	"	"	4.53
		8	3.500	"	"	5.74
		9	4.000	"	"	----
		10	0.000	"	"	3.42

*Standard reaction constituents, 0.5 gms. pancreatin, 3 cc. Na_2HPO_4 solution, 2 cc. of H_2O CO_2 free distilled, 0.5 cc. ethyl butyrate.

#No ethyl butyrate present. Controls to determine volatile fatty acids from cod liver oil film.

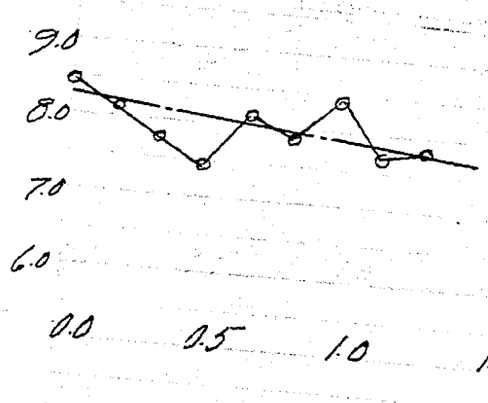
TABLE X (Cont'd)

Exp. No.	Fat or Oil	Flask No.	Gms. Fat or Oil Sand Prep.	Toluol Drops	Hrs. Incubation	Cc. of N/10 NaOH Required to Neut. Butyric Acid
		1	0.000	2	5½	4.21
		2	0.500	"	"	4.68
		3	1.000	"	"	5.10
	Wheat	4	1.500	"	"	4.92
67	Embryo	5	2.000	"	"	4.68
		6	2.500	"	"	5.03
		7	3.000	"	"	6.49
		8	3.500	"	"	5.50
		9	0.000	"	"	3.29
		1	0.000	5	5 2/3	8.48
		2	0.150	"	"	8.10
		3	0.300	"	"	7.77
	Cotton	4	0.450	"	"	7.45
39	Seed	5	0.600	"	"	8.17
	Oil	6	0.750	"	"	7.88
		7	0.90	"	"	8.40
		8	1.05	"	"	7.70
		9	1.20	"	"	7.82
		10	0.00	"	"	6.75
		1	0.000	2	6	3.60
		2	0.500	"	"	3.47
		3	1.000	"	"	4.00
	Glyce-	4	1.500	"	"	3.43
69	rol	5	2.000	"	"	3.90
		6	2.500	"	"	4.35
		7	3.000	"	"	5.43
		8	3.500	"	"	4.80
		9	0.000	"	"	3.00

TABLE X (Cont'd)

Exp. No.	Fat or Oil	Flask No.	Gms. Fat or Oil Sand Prep.	Toluol Drops	Hrs. Incubation	Cc. N/10 NaOH Required to Neut. Butyric Acid
		1	0.000	2	9	3.98
		2	0.500	"	"	3.21
		3	1.000	"	"	5.50
		4	1.500	"	"	5.10
68	Nujol	5	2.000	"	"	5.55
		6	2.500	"	"	5.10
		7	3.000	"	"	5.77
		8	3.500	"	"	5.68
		9	0.000	"	"	4.57
		1	0.000	4	6½	5.00
		2	0.500	"	"	5.18
		3	1.000	"	"	4.52
65	Olive	4	1.500	"	"	5.69
	Oil	5	2.000	"	"	4.91
		6	2.500	"	"	5.44
		7	3.000	"	"	4.76
		8	0.000	"	"	4.18

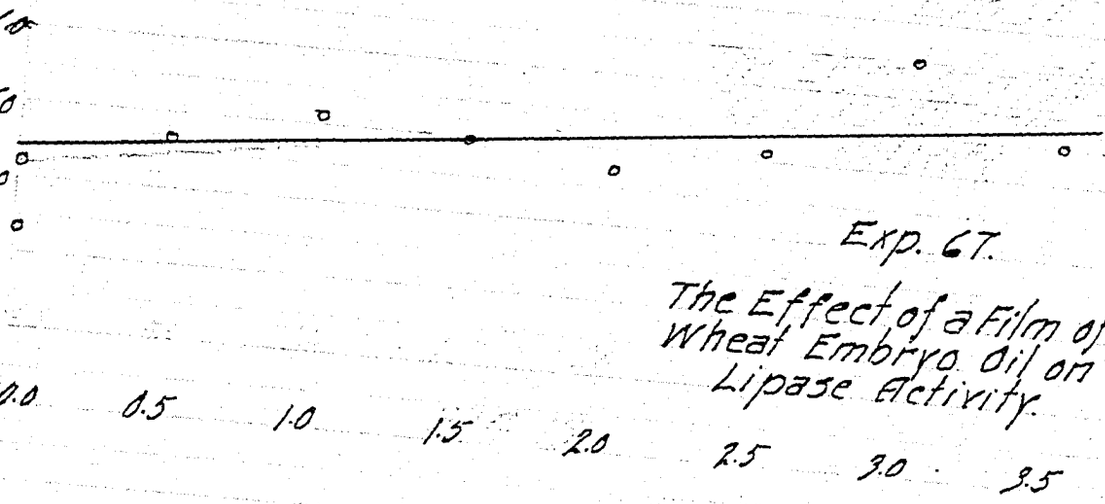
FIG. 5



Exp. 39.

The Effect of a Film of Cottonseed Oil on Lipase Activity.

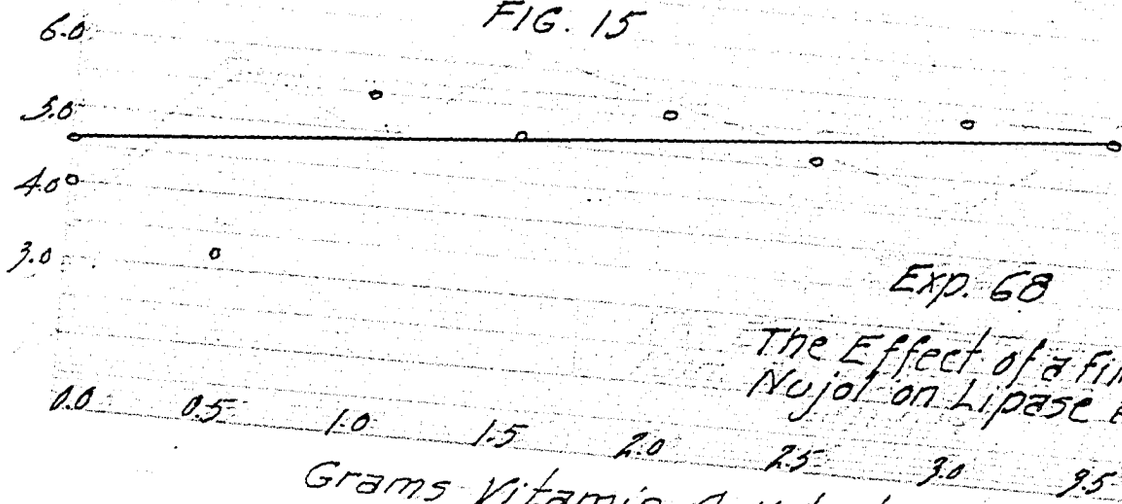
FIG. 12.



Exp. 67.

The Effect of a Film of Wheat Embryo Oil on Lipase Activity.

FIG. 15



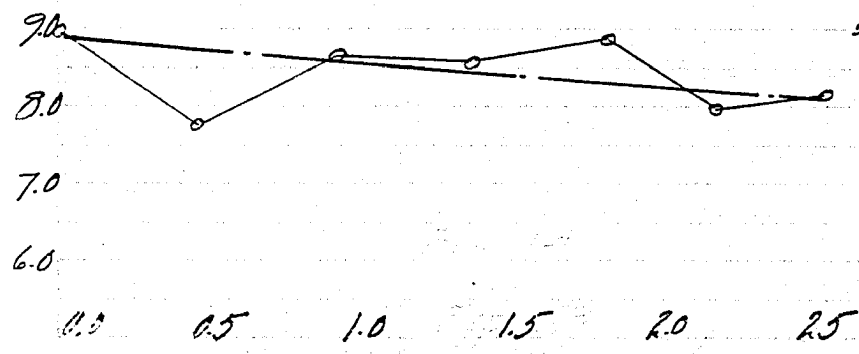
Exp. 68

The Effect of a Film of Nujol on Lipase Activity.

Milligrams of Olive Oil Neutralized by 1 c.c. Pancreatic Juice

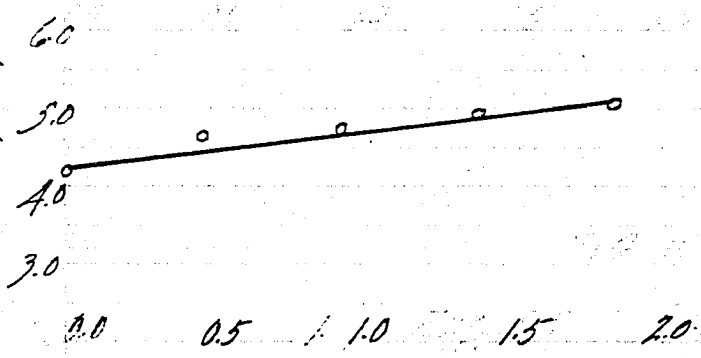
Grams Vitamin A, Vehicle

FIG. E1



Exp. 49.
The Effect of a Film of Butter Fat upon Lipase Activity

FIG. 12

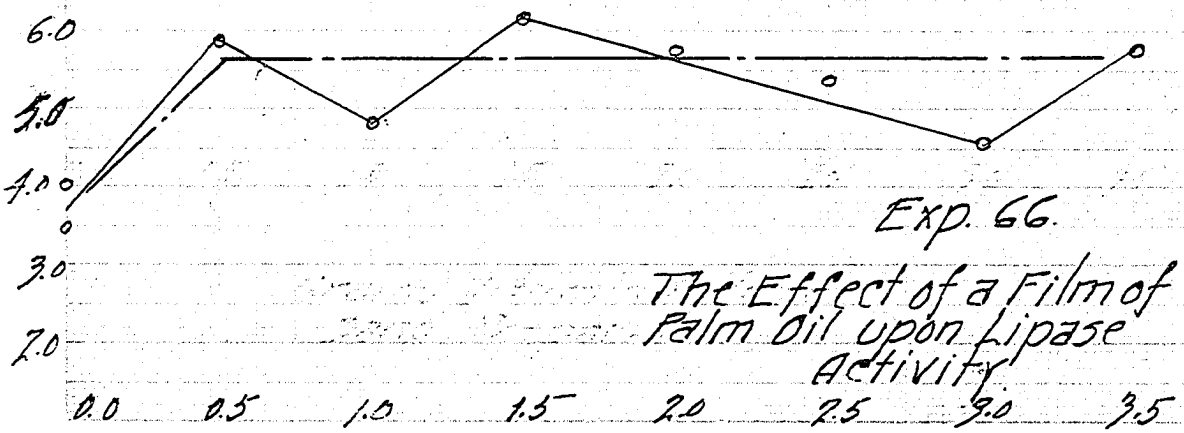


Exp. 51

The Effect of a Film of Cod Liver Oil upon Lipase Activity.

c.c. of N/10 NaOH

FIG. 13



Exp. 66.

The Effect of a Film of Palm Oil upon Lipase Activity

Grams Vitamin A Vehicle

FIG. 14

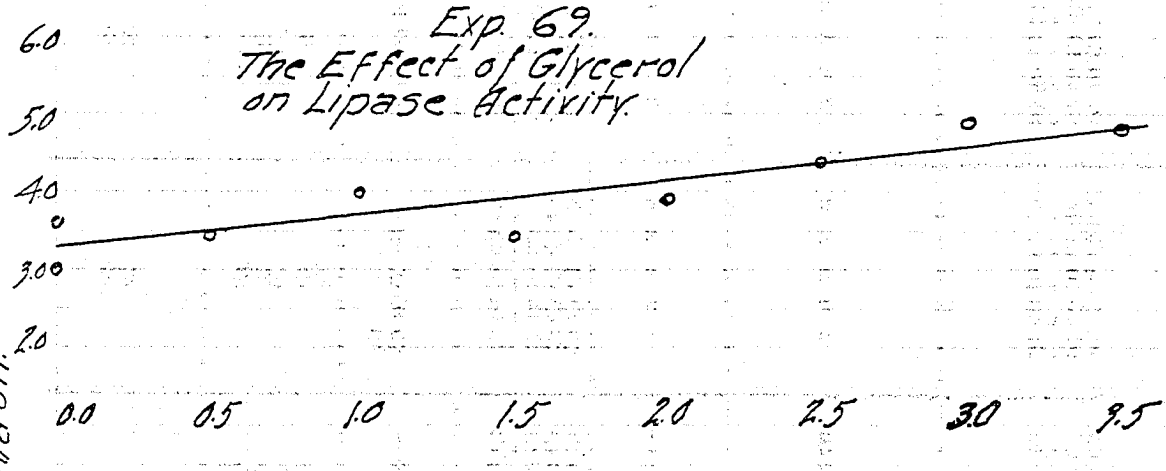

Exp. 69.
The Effect of Glycerol on Lipase Activity.
6.0
5.0
4.0
3.00
2.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
c.c. of N/10 NaOH.

FIG. 16

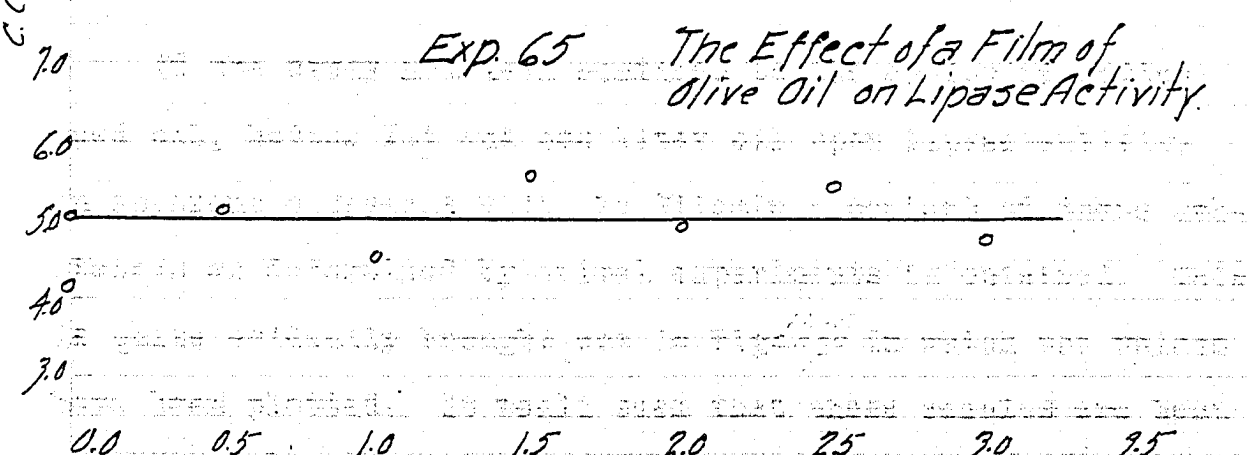

Exp. 65 The Effect of a Film of Olive Oil on Lipase Activity.
7.0
6.0
5.0
4.0
3.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Grams Vitamin A Vehicle Sand Preparation.

TABLE XI*

Exp. No.	Fat or Oil	Flask No.	Gms. Fat or Oil Sand Prep.	Toluol Drops	Hrs. Incubation	Cc. N/10 NaOH to Neut. Butyric Acid
78	Palm Oil	1	0.000	4	4½	2.40
		2	0.500	"	"	2.90
		3	1.000	"	"	2.85
		4	1.500	"	"	4.55
		5	2.000	"	"	2.89
79	Nujol	6	0.000	"	"	2.41
		7	0.500	"	"	2.59
		8	1.000	"	"	2.81
		9	1.500	"	"	3.20
		10	2.000	"	"	3.29
80	Wheat Embryo	1	0.000	4	5	2.33
		2	0.500	"	"	3.60
		3	1.000	"	"	3.65
		4	1.500	"	"	3.00

*Standard reaction constituents.

If the study had been confined to the action of cottonseed oil, butter fat and cod liver oil upon lipase activity an apparent agreement with the Vitamin A content of these substances as determined by animal experiments is obtained. This is quite evidently brought out in Figs. 11, 12 and 5 in which the values have been plotted. It would seem that these results are best explained by assuming that the activity noted is the resultant in each case of two factors--a depressing action of fats upon lipase and a stimulatory action on the part of the vitamin

FIG. 17

Exp. 78

The Depressing Action of Tolvol upon the Acceleration of Lipase caused by a Film of Palm Oil.

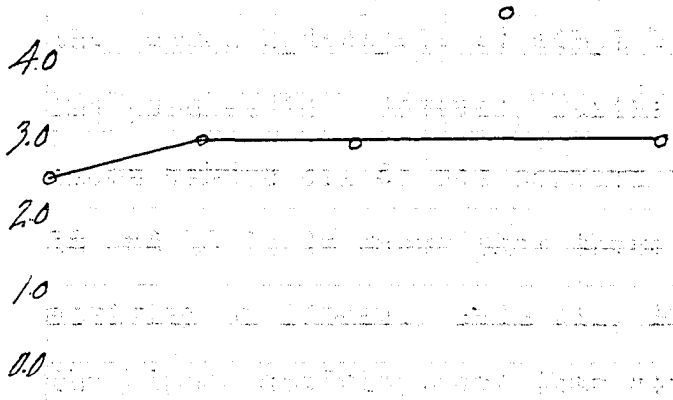


FIG. 18

Exp. 79
(Similar to Exp. 78.; Film is nujol.)

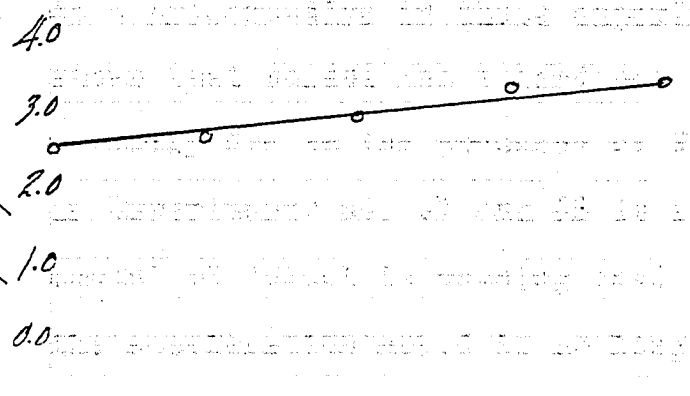
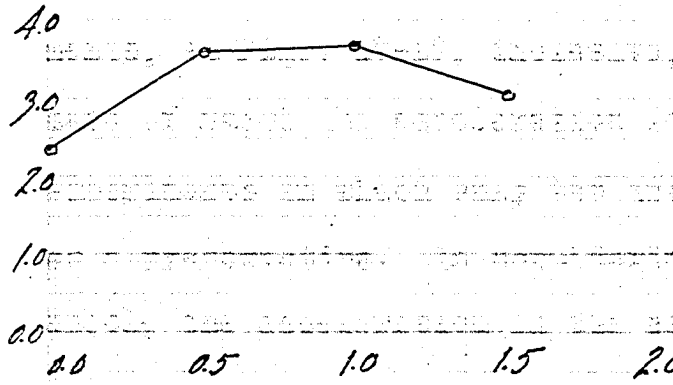


FIG. 19

Exp. 80

Similar to Exp. 78; (Film is wheat embryo oil).



Note: Compare with Figs. 13, 12, and 15.

Grams: Sand Film Prep.

present. In this connection reference is made to Experiment 41 in which it was shown that a film of oil tends to depress the normal hydrolysis of ethyl butyrate by the medium lacking pancreatin. However, further experiments with nujol and wheat embryo oil do not confirm this assumption. In Figures 12 and 15 it is shown that these substances accelerate the activity of lipase. Palm oil, Exp. 66, Fig. 13, accelerated the lipase activity more than cod liver oil, which is known to be one of the richest sources of Vitamin A. Attention is called to the slight difference in the amount of toluol used as a preservative in these experiments. In Exp. 40 it was shown that toluol has a tendency to depress the acceleration normally due to the presence of Vitamin A concentrate. Thus, in Experiments 66, 67 and 68 it is probable that the smaller amount of toluol is causing enuf change in the medium that the acceleration noted is no longer comparable to the results obtained in similar experiments with other fats or oils but with a slightly higher toluol content. It seemed worth while to test this point. In Table XI is presented data in support of this contention. Reference to the plots for these experiments, in Figs. 17-19, inclusive, shows that except in the case of nujol the acceleration is much less than in those experiments in which only two drops of toluol had been used as a preservative. In considering the chemical nature of nujol, the acceleration in the case of a film of nujol is

highly interesting but is not serious evidence against the idea that Vitamin A has stimulatory action upon lipase.

D. The Effect of Vitamin A Concentrates made from other Sources than Butter Fat.

The acceleration noted in Experiments 19, 28 and 29 was caused by the addition of Vitamin A concentrate. Similar acceleration should be noted in case of concentrates made from cod liver oil and alfalfa as both of these substances have been shown by animal experiments to be rich in Vitamin A. However, such concentrates made from lard, cottonseed oil, and inactivated butter fat should prove not to stimulate lipase activity. This reasoning has been tested out. The experimental data is shown in Table XIII and Figures 20-27, inclusive.

The concentrates from these sources were made in the manner previously indicated. The yields are summarized below in Table XII.

Table XII

Source of Vitamin A	Gms. Fat or Oil Saponified	Gms. Extract
Cod liver oil	300	2.9942
Lard	300	1.6904
Cottonseed oil	300	2.0446
Inactivated butter fat	260	2.1457
Alfalfa	*	8.9616

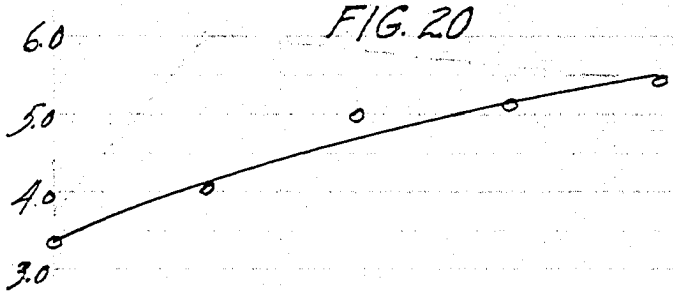
*2578 gms. alfalfa extracted with 95% C_2H_5OH.

TABLE XIII*

Type of Vitamin A Prep.	Gms. Sand Prep.	Cc. of N/10 NaOH to neut. Butyric Acid: Cod Liver Oil Vit. A Prep.	Cotton-seed oil Vit. A Prep.	Lard Vit. A Prep.	Inact. B.F. Vit. A Prep.
Exp. No.		55	54	56	53
Incubation		4	4	4	4
Flask No.					
1	0.000	3.38	3.92	3.03	3.41
2	0.500	4.06	3.90	3.80	4.90
3	1.000	5.00	5.31	5.09	4.80
4	1.500	5.15	4.81	4.20	4.20
5	2.000	5.44	5.95	3.98	4.18
Exp. No.		63	59	61	60
Incubation		9½	10½	10	5½
Flask No.					
1	0.00	4.80	4.87	5.20	4.52
2	0.50	7.00	7.08	7.95	6.98
3	1.00	6.06	6.06	7.05	4.57
4	1.50	6.50	--	7.90	5.90
5	2.00	6.50	6.08	8.25	6.10
6	0.00	5.42	4.45	5.40	4.02
7	2.50	6.20	6.85	7.40	4.79
8	3.00	6.10	6.48	7.45	5.10
9	3.50	5.80	5.73	7.75	6.33
10	0.00	4.40	4.22	5.20	3.19

*Standard reaction constituents used.

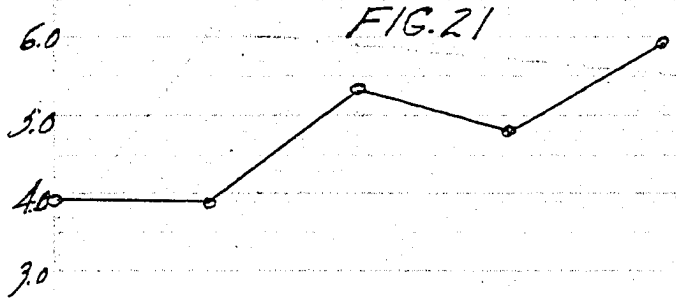
FIG. 20



Exp. 55

The Effect of Vitamin A Concentrate made from Cod Liver Oil upon Lipase Activity

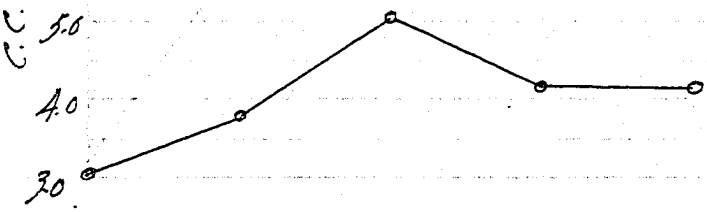
FIG. 21



Exp. 54

The Effect of Vitamin A Concentrate made from Cottonseed Oil upon Lipase Activity.

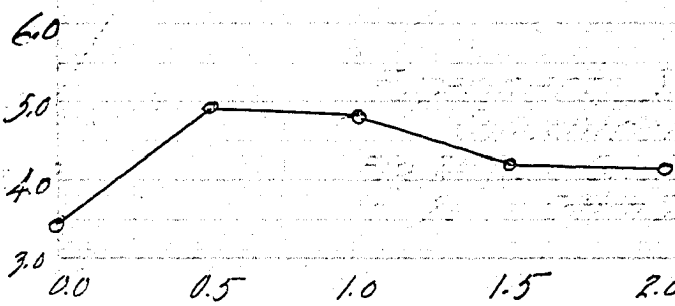
FIG. 22



Exp. 56

The Effect of Vitamin A Concentrate made from Lard upon Lipase Activity

FIG. 23



Exp. 57

The Effect of Vitamin A Concentrate made from Inactivated Butter Fat upon Lipase Activity.

FIG. 24

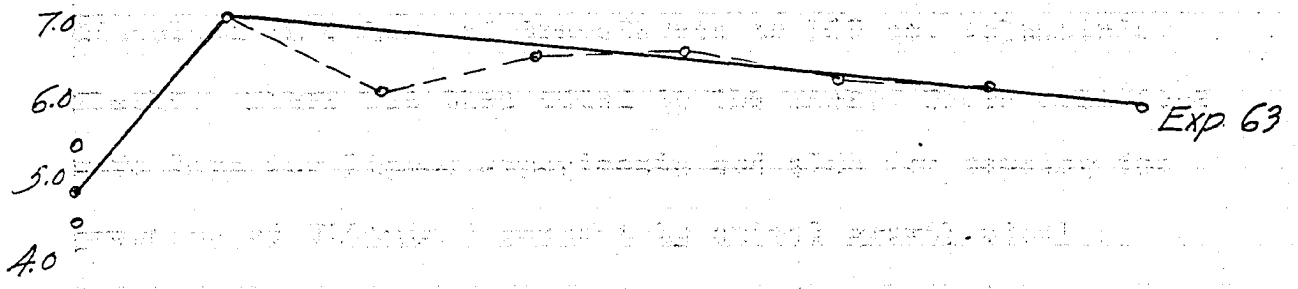


FIG. 25

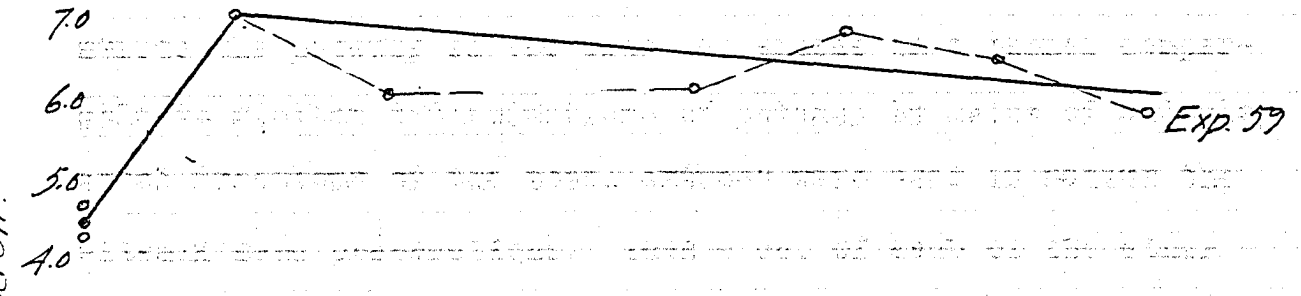


FIG. 26.

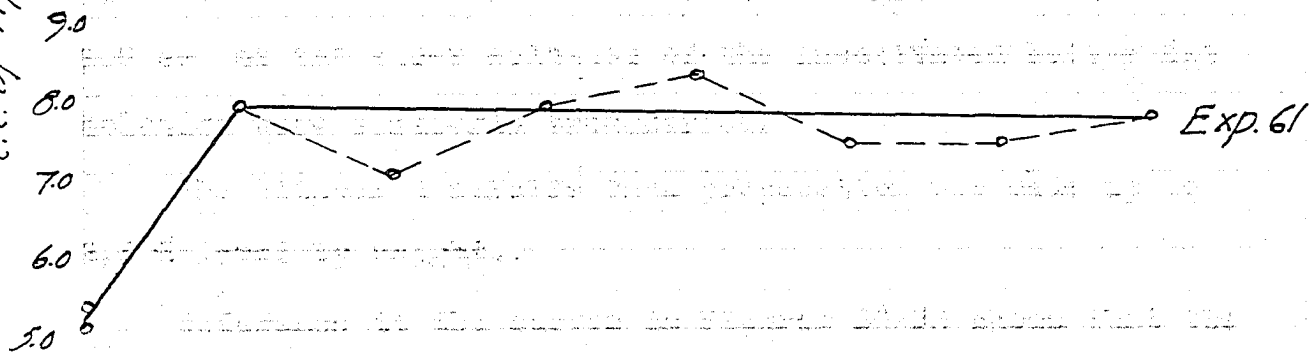
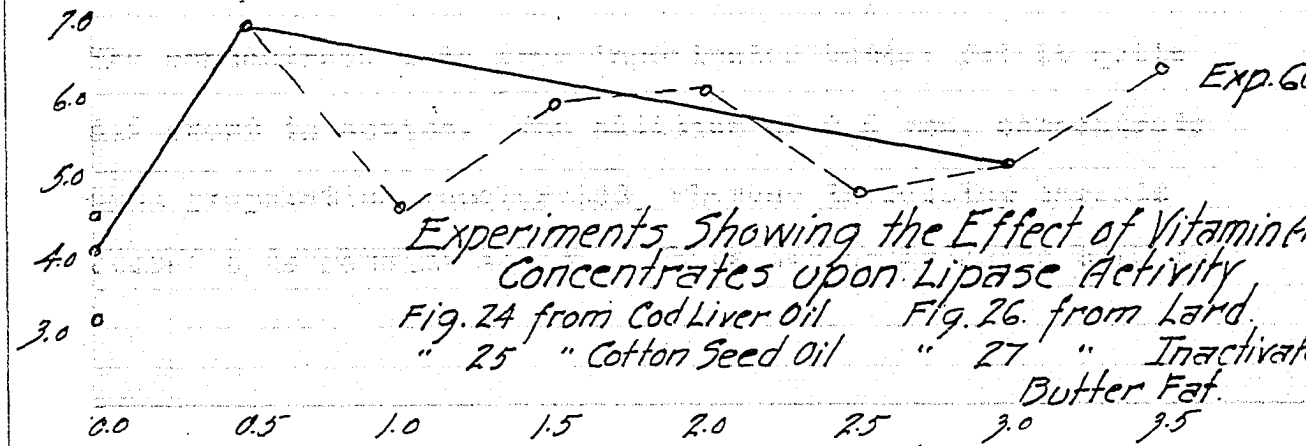


FIG. 27.



Experiments Showing the Effect of Vitamin A Concentrates upon Lipase Activity.

Fig. 24 from Cod Liver Oil Fig. 26. from Lard.
" 25 " Cotton Seed Oil " 27 " Inactivated Butter Fat.

The concentrates made from the first four sources were dissolved in ether and transferred to 100 cc. volumetric flasks. Ether was then added to the mark. These solutions were kept for lipase experiments and also for testing for presence of Vitamin A content by animal growth studies.

In the making of the vitamin sand preparations, the level of Vitamin A concentrate was maintained as close as possible at 2.5% by weight of the final product. To demonstrate the potency of the original source in a manner comparable to similar determinations of potency by means of animals, equal fractions of the total extract were used in making the vitamin sand preparations. Thus 6 cc. of each of the ether solutions of the concentrates made from cod liver oil, lard and cottonseed oil were transferred to 8 grams of sand. 6.9 cc. of the ether solution of the inactivated butter fat solution were similarly transferred.

The Vitamin A alfalfa sand preparation was made up to 2.5 % level by weight.

Reference to the curves in Figures 20-23 shows that the cod liver oil concentrate accelerated lipase activity in much the same manner as a similar concentrate made from butter fat. The concentrate made from inactivated butter fat is quite different in action. The addition of 0.5 gms. concentrate sand preparation accelerated, whereas increasing amounts caused a depressing action. These two experiments seem to

indicate that the cause of the acceleration lies in the Vitamin A content. Opposing these two experiments, however, are Experiments 54, 56, 63, 59, and 61, Figures, 21, 22, 24, 25, and 26, respectively. In Fig. 21 and 22 it is seen that concentrates made from cottonseed oil and lard also cause an acceleration. The curves are, however, irregular. This irregularity was the cause of an attempt to duplicate the results with these concentrates. Figures 24-27, inclusive, indicate the results obtained. Only in the case of Exp. 53 is it safe to infer that duplication was obtained. It is to be noted, however, that, in respect to Exps. 53 and 60, the time element remained practically the same; whereas in Exps. 63, 59 and 61 the time interval had been increased a little more than two times what it was in Exps. 55, 54, 56 and 53. The data is not therefore on a strictly comparable basis.

Experiment 48, Table XIV, shows the effect of a Vitamin A concentrate made from alfalfa upon lipase activity. This concentrate was made according to the directions of Steinbock and Boutwell[14]. Approximately 2500 grams of dry powdered alfalfa meal of good quality was twice extracted with cold 95 percent alcohol. The alcohol extract was concentrated by distilling off the alcohol. Excess of 20 percent alcoholic potash was then added. The saponification mixture then stood for four days after which it was shaken with ether. This product was refined according to Steinbock and Boutwell's proce-

dure. The petroleum ether layer which has been shown by these authors to be potent in Vitamin A was used in the tests with lipase.

During the production of this concentrate the author had to allow the saponified mass to stand for four days at room temperature. Ample opportunity was therefore afforded for destruction of Vitamin A. That destruction took place was shown by animal experiments. It can also be seen from Fig. 28 that the lipase is not accelerated in the same manner as with concentrates known to contain Vitamin A.

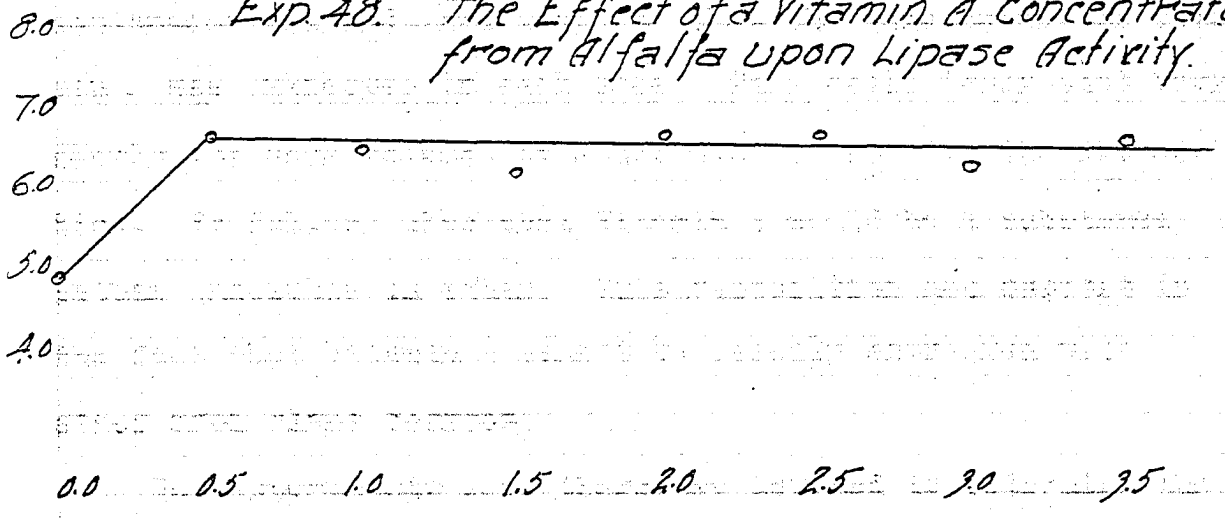
TABLE XIV*

Flask No.	Gms. Vit. A Sand Prep.	Cc. N/10 NaOH to neut. Butyric Acid
1	0.000	4.81
2	0.500	6.60
3	1.000	6.44
4	1.500	6.21
5	2.000	6.60
6	0.000	4.38
7	2.500	6.60
8	3.000	6.10
9	3.500	6.50
10	0.000	4.50

*Standard reaction constituents, 4 drops toluol. Incubation period 7¾ hours.

FIG. 28

Exp. 48. The Effect of a Vitamin A concentrate from Alfalfa upon Lipase Activity.



Note: This concentrate shown by animal growth to be deficient in Vitamin A.

E. Rat Growth Studies on Vitamin A. Potency of Concentrates from Cod Liver Oil, Cottonseed Oil, Lard, Inactivated Butter Fat and Alfalfa.

The acceleration of lipase activity caused by concentration from cod liver oil, cottonseed oil, and lard, as shown in Figures 20-22, inclusive, may be assumed to be due to Vitamin A if these concentrates permitted animal growth at about the same rate. The concentrate from alfalfa could also be tested with animal growth to determine its potency.

The only condition under which the rate of animal growth would be the same for concentrates made from cod liver oil, cottonseed oil and lard would be that the same amount of Vitamin A was extracted in each case. This would imply that very nearly the same volumes of ether must be used in the extraction. It follows also that Vitamin A would be a substance rather insoluble in ether. This supposition has support in the fact that Vitamin A cannot be readily extracted with ether from plant sources.

Rat experiments were therefore devised to determine the accuracy of this reasoning. Rats were fed a deficient diet until their growth was stationary, when concentrates from the various sources were added to the ration. The amount of concentrate was based upon 5% level of the original source. Control rats, except for alfalfa and inactivated butter fat, were fed the deficient ration plus 5% of the original source.

The results obtained from these experiments have been incorporated into Table XVI and the growth curves are presented in Figures 29-37, inclusive.

In Table XV is presented the volume of the ether solutions of Vitamin A concentrates used to supplement the deficient rations in the rat experiments.

TABLE XV

Concentrate	Date in Ration	Cc. per 1000 gms. Ration	Gms.	Date in Ration	Cc. per 200 gms. Ration	Gms.
Cottonseed oil	7/7/25	16.5	0.3374	--	--	--
Inactivated butter fat	7/7/25	19.2	0.4119	--	--	--
Lard	7/7/25	16.5	0.2788	--	--	--
Cod liver oil	7/7/25	16.5	0.4940	8/6/25	*16.5	0.3374
Alfalfa	7/7/25	--	0.1755	8/6/25	--	0.100

*Added to 200 gms. of ration to which previously had been added 0.3374 gms. per 1000 gms. ration.

It can be seen from the growth curves that only the concentrate from cod liver oil permitted animal growth. Steinbock, Sell, and Buell[14] reported that a similar concentrate from butter fat gave growth. Addition of concentrate equivalent to a 5% level of the original source did not improve those rations supplemented by concentrates from inactivated butter fat, lard, cottonseed oil and alfalfa. The effect of

such addition was considerable in the case of the ration supplemented by cod liver oil concentrate. The amount of cod liver oil concentrate and also of alfalfa concentrate was increased later to approximately 20 percent. The rats getting the additional amount of Vitamin A cod liver oil concentrate did very well, whereas this cannot be said of the rats getting the concentrate from alfalfa.

TABLE XVI

Vitamin A Vehicle	Butter Fat					Cottonseed Oil			
Rat No.	21	22	23	24	25	26	27	28	29
Sex	♀	♀	♂	♂	♂	♂	♀	♀	♀
5/29/25	60	61	71	61	69	69	61	61	55
6/5	70	75	75	70	80	80	70	76	62
6/13	91	80	78	94	95	93	79	81	73
6/19	99	82	95	85	101	95	80	97	75
6/29	102	78	96	87	105	106	82	100	76
7/7	106	80	93	73	104	99	77	104	70
7/14	117	89	97	72	111	#104*	85	*101	#68
7/26	140	110	109	84	141		*90	#90	
8/5	159	119	149	109	167		@77		
8/18	170	151	148	133	186				

Vitamin A Vehicle	Cod Liver Oil					Lard¢				
Rat No.	30	31	32	33	34	35	36	37	38	39
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♀	♀
5/29/25	56	65	61	52	61	59	61	57	62	77
6/5	72	80	74	70	64	69	66	60	69	87
6/13	88	93	80	86	69	74	73	65	75	88
6/19	98	96	99	93	72	86	76	76	75	93
6/29	99	104	113	103	85	86	66	66	76	90
7/7	139	121	144	126	123	@70	57	60	72	*84
7/14	158	127	156	129	143		&	%63	*76	*84
7/28	193	143	179	173	176	(7/20)			73	*81
						(7/28)			#	#
8/5	215	163	194	150	190					
8/18	206	170	188	167	189					

*Eyes sore.
@Killed.
#Found dead.
&Escaped.
%Killed, ear infection.
¢Heavy line indicates addition of an amount of concentrate equivalent to 5% of the original source of the concentrate.

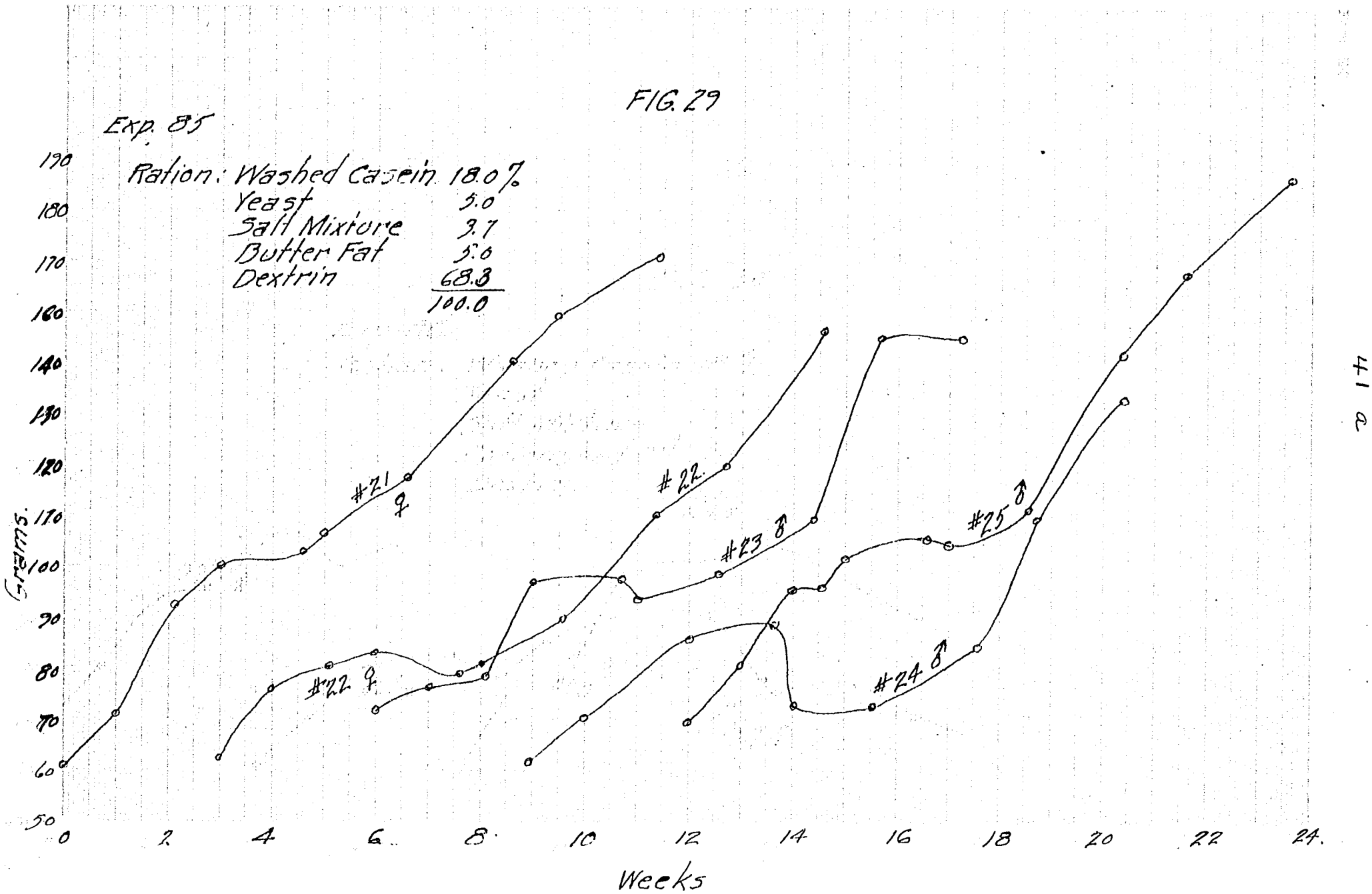
TABLE XVI (Cont'd)

Vitamin A Vehicle	Inactivated Butter Fat Conc.¢					Cottonseed Oil Conc.¢				
Rat No.	1	2	3	4	5	6	7	8	9	10
Sex	♂	♀	♂	♀	♀	♀	♂	♂	♂	♀
5/29/25	60	59	59	56	60	64	67	71	60	61
6/5	69	68	73	61	72	70	78	79	72	95
6/13	79	77	78	66	78	74	83	86	86	94
6/19	83	80	78	68	81	77	90	92	92	98
6/29	75	86	66	66	74	76	91	90	96	96
7/3	72	84	65	72	77	78	91	68	97	96
7/14	67	78*	#	66@	72	#	79#	60*	78*	84
7/20	#	83		#	72			#	#	75
7/28		65@			#					59#

Vitamin A Vehicle	Cod Liver Oil Conc.¢					Lard Conc.¢				
Rat No.	11	12	13	14	15	16	17	18	19	20
Sex	♀	♂	♀	♀	♀	♂	♀	♂	♀	♂
5/29/25	64	56	61	59	70	63	59	58	50	68
6/5	74	67	67	68	81	78	80	67	50	80
6/13	86	77	73	70	85	89	90	66	61	89
6/19	98	95	71	81	94	100	96	67	66	97
6/29	99	97	64	77	91	101	100	62	66	103
7/3	100	101	53	73	91	102	101	62	68	105
7/14	110	101	50	77	101	92	94*	67	63*	107
7/20	106	122	48	78*	115	77	89	64	64*	104
7/28	88%	111	42@	84&	117	60	85#	66@	62#	85#
8/5		121		105	146	#				
8/14		137		121	144					

*Eyes sore.
@ Killed.
# Found dead.
% Killed, ear infection.
¢ Heavy line indicates addition of an amount of concentrate equivalent to 5% of the original source of the concentrate.
& Eyes well.

FIG. 29


Exp. 85
Ration: Washed Casein 18.0%
Yeast 5.0
Salt Mixture 3.7
Butter Fat 5.6
Dextrin 68.3
100.0
#21 ♀
#22 ♀
#22.
#23 ♂
#24 ♂
#25 ♂
Grams
Weeks
190
180
170
160
140
130
120
110
100
90
80
70
60
50
0
2
4
6
8
10
12
14
16
18
20
22
24

41 a

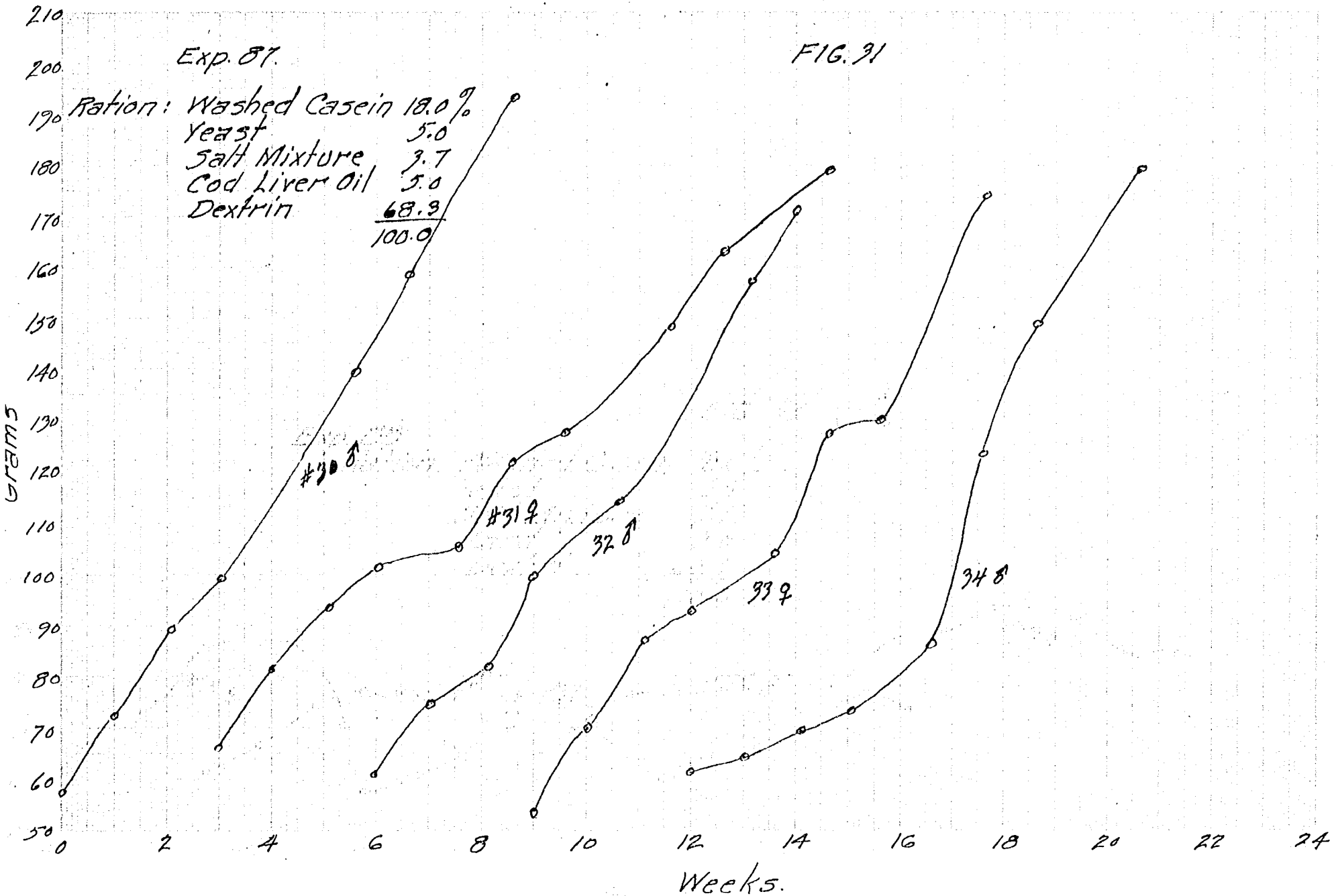

Exp. 87.
FIG. 31
Ration: Washed Casein 18.0%
Yeast 5.0
Salt Mixture 3.7
Cod Liver Oil 5.0
Dextrin 68.3
100.0
#30 ♂
#31 ♀
32 ♂
33 ♀
34 ♂
Grams
Weeks.
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
0
2
4
6
8
10
12
14
16
18
20
22
24

FIG. 32.

Exp. 88
Ration. Washed Casein 18.0%
Yeast 5.0
Salt Mixture 3.7
Lard 5.0
Dextrin 68.3
100.0

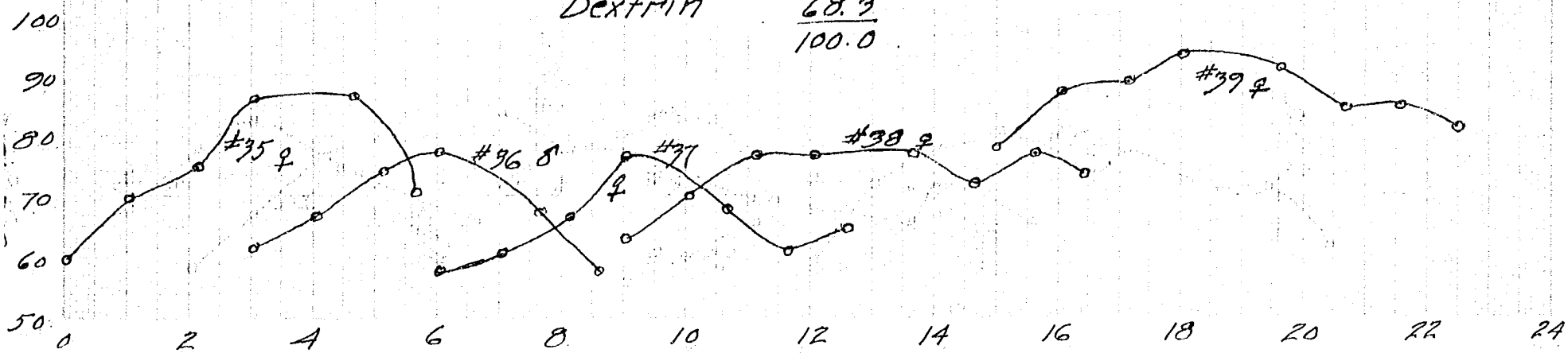


FIG. 33

Exp. 81.

Ration: Washed Casein 18 %
Yeast 5
Salt Mixture 3.7
Dextrin 73.3
100.0

Supplemented 7/7/25 by 19.2 cc of ether solution of Vitamin A concentrate made from inactivated butterfat.

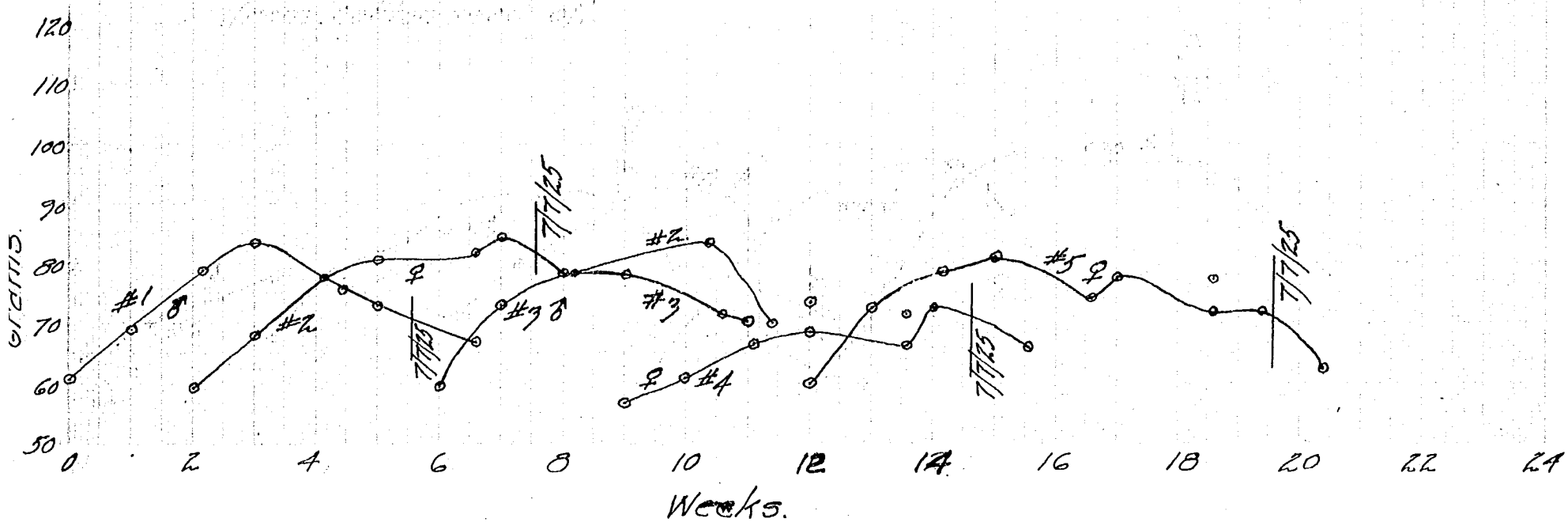


FIG. 34

Exp. 82.

Ration: Washed Casein 18.0 %
Yeast 5.0
Salt Mixture 3.7
Dextrin 73.3
100.0

Supplemented 7/7/25 by 16.5 cc of ether solution of Vitamin A concentrate made from cottonseed oil.

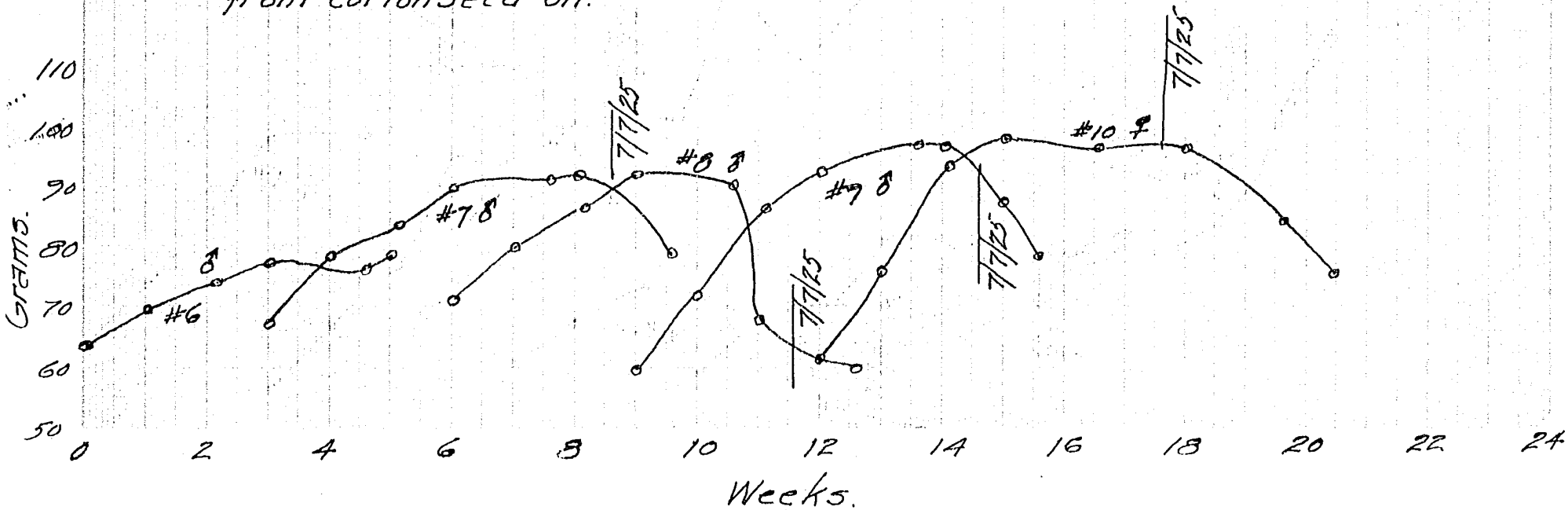


FIG. 35

Exp. 83.

Ration:		
	Washed Casein	18.0 %
	Yeast	5.0
	Salt Mixture	3.7
	Dextrin	73.3
		100.0

Supplemented 7/7/25 by 16.5 c.c. ether solution of Vitamin A concentrate made from cod liver oil

Supplemented 8/6/25 by 82.5 cc of same solution.

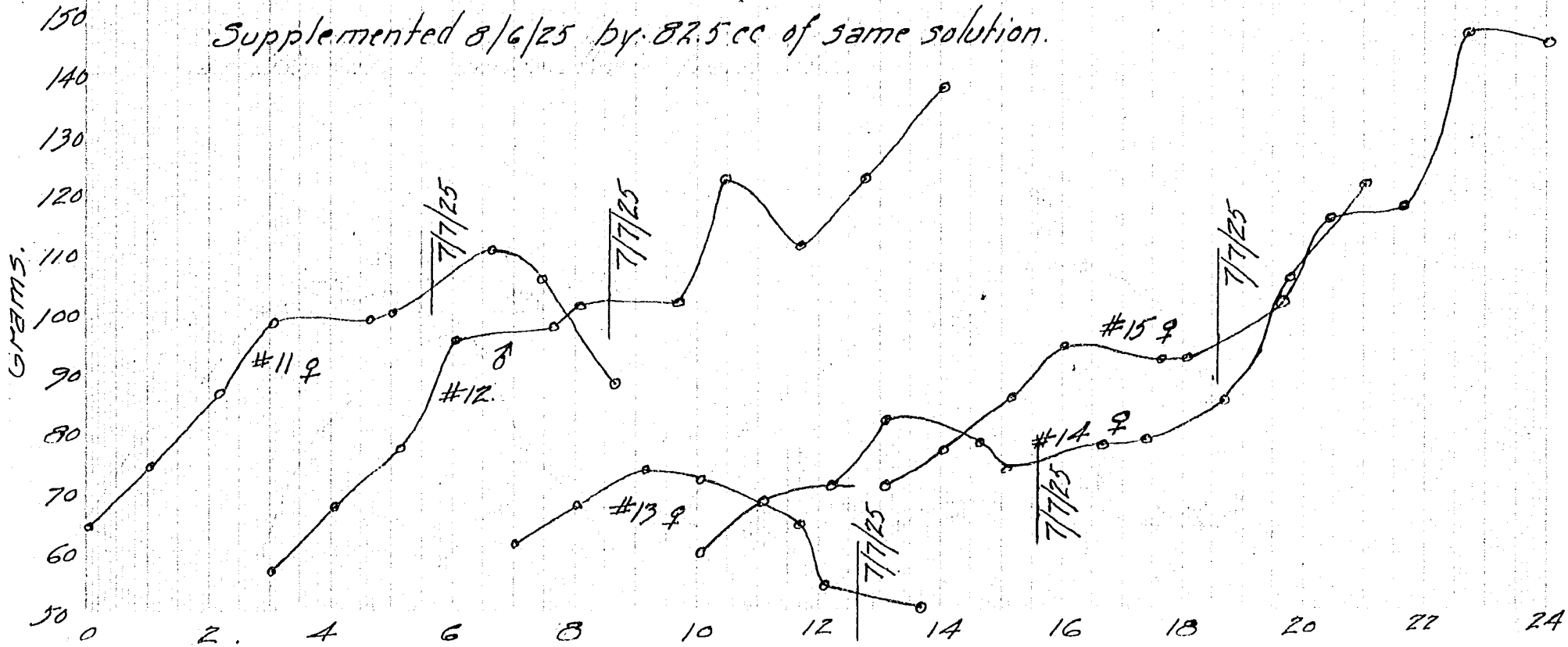


FIG. 36

Exp. 84.

Ration : Washed Casein 18.0%
Yeast 5.0
Salt Mixture 3.7
Dextrin 73.3
100.0

Supplemented 7/7/25 by 16.5 c.c. of ether solution of Vitamin A concentrate from lard.

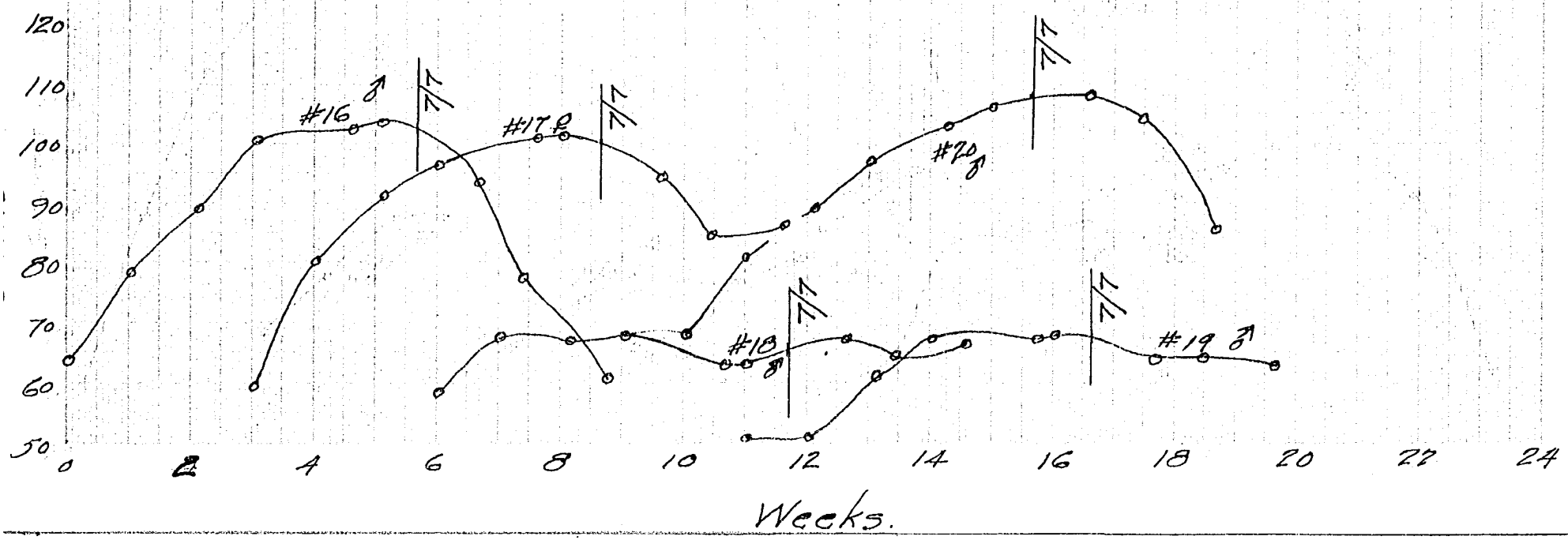


FIG. 37

Exp. 89. Ration:

Washed casein	18.0%
Yeast	5.0
Salt Mixture	3.7
Dextrin	73.3
	100.0

Supplemented 7/7/25 by 0.1753 gms. Vitamin A concentrate made from alfalfa.

Supplemented 7/28/25 by 5 times the amount of concentrate given 7/7/25.

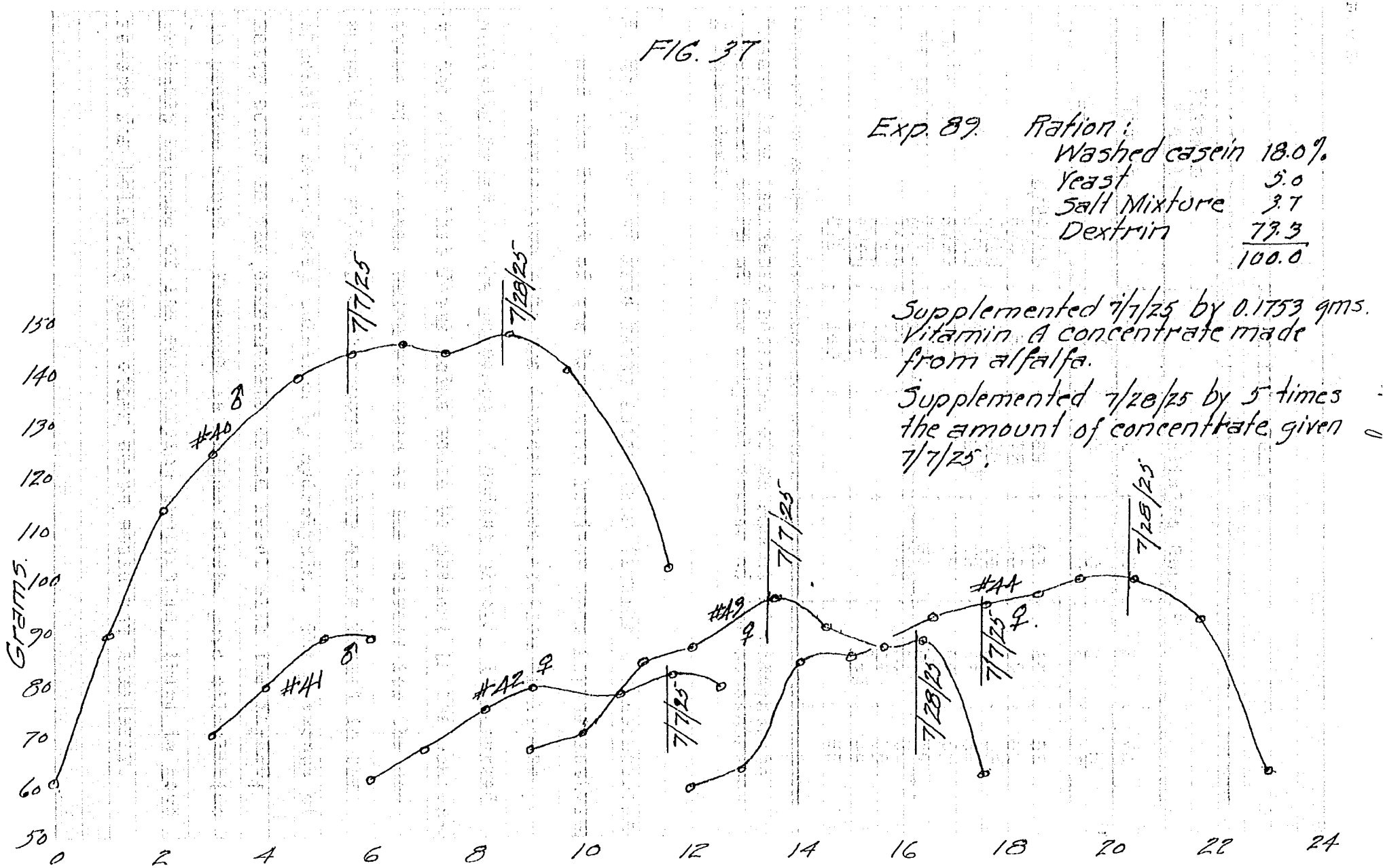


TABLE XVI (Cont'd)

Vitamin A Vehicle	Alfalfa Conc.*				
Rat No.	40	41	42	43	44
Sex	♂	♂	♀	♀	♀
5/29/25	60	69	61	67	59
6/5	88	73	66	69	62
6/13	113	88	74	84	83
6/19	124	88#	78	86	84
Date of 6/29	138		77	96	92
Weighing: 7/7	143		81	90	94
7/14	144		78#	86	96
7/20	143			87	99
7/28	147			62#	99
8/5	140				92

*Heavy line indicates addition of an amount of concentrate equivalent to 5% of the original source of the concentrate.
Found dead.

F. The Removal of Free Fatty Acids from Vitamin A Concentrates and the Effect of the Resulting Product upon Lipase Activity.

In connection with the decided acceleration of lipase activity noted in the presence of sodium oleate, it occurred to the author that fatty acids would be present in the concentrate of the ether which was used as the solvent for the unsaponifiable matter, also removed fatty acids formed in the alcoholic water soap solution by hydrolysis. The presence of fatty acids in the concentrate would react with the Na_2HPO_4 to produce soaps. Inasmuch as sodium oleate gave stimulation probably because of its emulsifying properties, there is good reason for believing that other soaps would act similarly.

Experimental data shows that fatty acids may be extracted quite readily from soap solutions by means of ether. J. Lewkowitsch[15] states that palmitic acid has thus been extracted from a sodium palmitate solution by toluene. 0.0319 grams palmitic acid (Kahlbaum) was dissolved in 95% ethyl alcohol. 10 cc. of distilled water was then added. This solution was basic when tested with phenolphthalein. This solution was extracted three times with ether. The combined ether extracts were washed with distilled water five times, the last three washings of which were done with CO_2 free distilled water. There remained a residue after removing the ether by evaporation. This residue required 1.22 cc. of N/10 NaOH for neutralization. The various concentrates were all found to be decidedly acid in character when tested with N/10 NaOH, performing the titration in alcohol as solvent for the concentrate.

The elimination of fatty acids from the Vitamin A concentrate has proved to be a difficult task because of the solubility of soaps in the solvents used for the unsaponifiable matter. The author was able to effect a rather complete separation of the fatty acids from the concentrate from butter fat. The final product required an insignificant amount of N/10 NaOH for neutralization and gave no test for potassium using $NaCO(NO_2)_6$. The resulting product accelerated lipase activity decidedly. Hence it seems that the acceleration is

due in part only to the presence of soaps. The data for this experimental work is presented in Table XVII.

TABLE XVII*

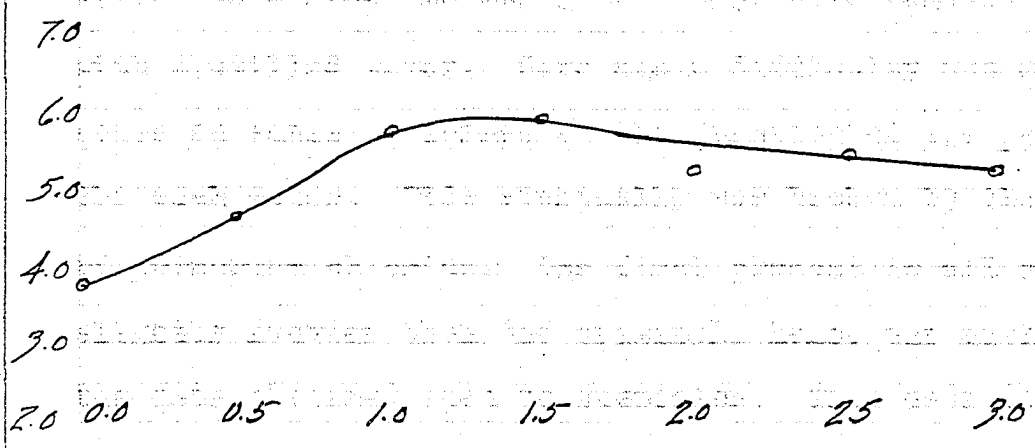
Flask No.	Gms. Vit. A B.F. (purified) Sand Prep.	Cc. N/10 NaOH to neutralize Butyric Acid
1	0.000	3.70
2	0.500	4.55
3	1.00	5.65
4	1.50	5.80
5	2.00	5.20
6	2.50	5.40
7	3.00	5.20

*Standard reaction constituents, 2 drops toluol. Incubation period 5¾ hours.

The removal of free fatty acids was effected by first neutralizing the fatty acids in the concentrate by the addition of alcoholic potassium hydroxide solution to the concentrate dissolved in alcohol. The amount of alcoholic potassium hydroxide necessary had previously been determined by using smaller amounts of concentrate. A slight excess of potassium hydroxide was deemed advisable and so used. The alcohol used as solvent for both potassium hydroxide and concentrate was absolute. Under these conditions E. B. Holland et al[16] claim that the reaction is complete. The alcohol was then removed by exhaustion with a high vacuum pump. The soaps were then extracted with ether. The ether extract proved, however, to contain large amounts of soaps. By changing the solvent from di-

FIG. 38

Exp. 64. The Effect of Vitamin A (D.F.) Concentrate (free from fatty acids) upon Lipase Activity.



Grams Vitamin A concentrate (free from fatty acids) sand preparation.

ethyl ether to petroleum ether of low boiling point it was possible to obtain, after three fractionations, an extract very free from soaps and crystalline in character. This product was used in testing lipase activity.

A direct relationship between the animal growth and the lipase acceleration experiments would be established if it could be shown that the concentrates from cottonseed oil, lard and inactivated butter fat do not accelerate when free from fatty acids. The establishment of this relationship was attempted but unfortunately the purification of the concentrates proved difficult. The soaps persisted in being dissolved in the petroleum ether. A great deal of soap was removed, however. As a final measure, the soaps were removed by washing with distilled water. Here again difficulty was met with because an emulsion formed at the junction of the petroleum and the wash water. This eventually was broken by the addition of potassium chloride. The final product in all cases proved slightly heavier than the original, hence the author deems the data obtained open to suspicion. This data is presented in Table XVIII.

TABLE XVIII*

Type of Vit. A Sand Prep.		Cod Liver Oil	Cotton-Seed Oil	Lerd	Inactivated Butter Fat
Exp. No.		74	77	76	75
Flask No.	Gms. Sand Prep.	Cc. of N/10 NaOH			
1	0.000	2.86	2.55	--	2.81
2	0.500	3.25	--	3.50	3.93
3	1.000	3.40	3.60	4.12	3.65
4	1.500	3.30	3.60	--	4.40

*Reaction Constituents:

0.5 gms. pancreatin

0.5 cc. ethyl butyrate

5.0 cc. H_2O distilled CO_2 free

5.0 cc. Na_2HPO_4 Sol.

4 drops toluol

V. Summary.

1. A method to study the effect of Vitamin A concentrates upon the enzyme lipase has been devised.
2. Several phases of this method have been studied to eliminate possible sources of acceleration other than that caused by Vitamin A or similar concentrates.
3. An acceleration of lipase activity by Vitamin A concentrates has been noted and studied with the view to correlating this acceleration with the Vitamin A content of various fats and oils.
4. Concentrates made from butter fat and cod liver oil have been found to accelerate lipase activity. A concentrate from inactivated butter fat was found to give very little acceleration.
5. Concentrates made from cottonseed oil and lard were found to accelerate lipase activity. These concentrates, however, have been shown to contain fatty acids which react with the medium to give soaps.
6. Sodium oleate has been found to accelerate lipase activity.
7. An alfalfa concentrate shown to be deficient in Vitamin A did not accelerate lipase activity.
8. A Vitamin A concentrate from butter fat has been separated from its free fatty acid content and found to give acceleration.

9. Vitamin A vehicles such as butter fat, cod liver oil, palm oil, cottonseed oil, lard, olive oil and wheat embryo oil when tested for lipase acceleration are not in all cases in accord with the potency of these sources as determined by animal growth.
10. A Vitamin A concentrate has been obtained which is free from fatty acids.
11. It has been found that the separation of unsaponifiable matter from soaps is not reliable.

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