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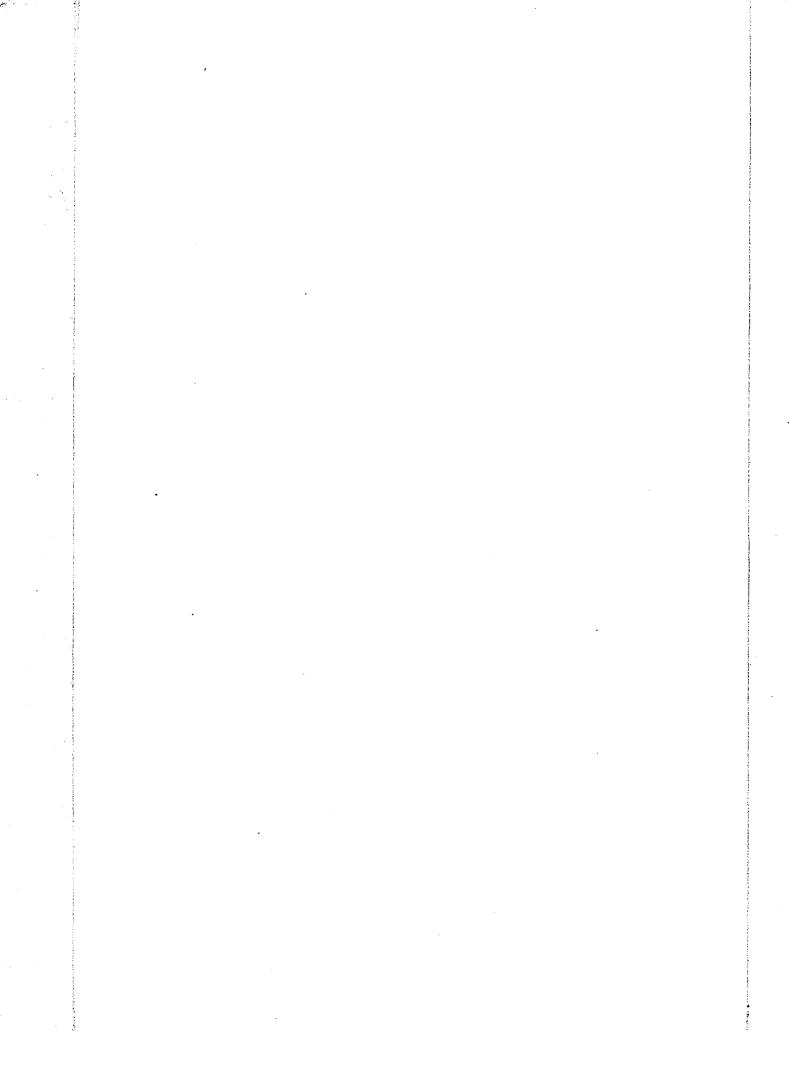
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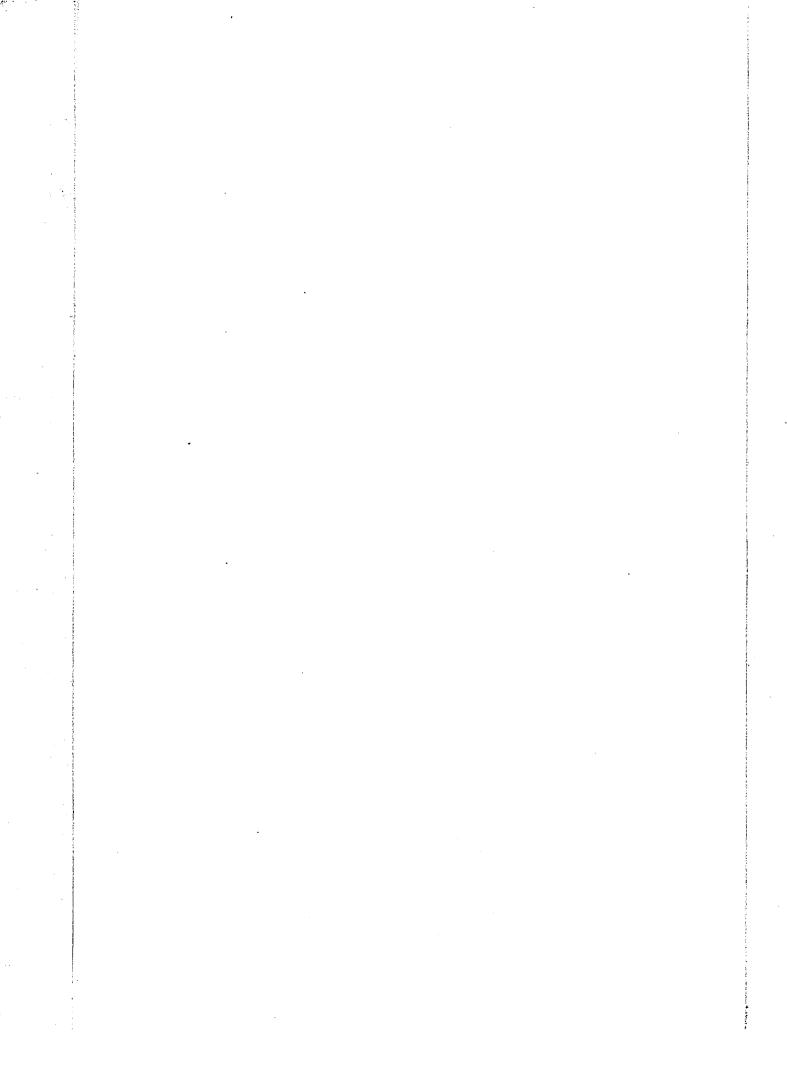
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ON THE ACCELERATION OF LIPASE ACTIVITY CAUSED BY THE ACTION OF SUBSTANCES CONTAINING VITAMIN A

By

Birger L. Johnson

A Thesis

Submitted to the Graduate Faculty for the Degree of

Doctor of Philosophy

Major Subject: Chemistry

Approved

Signature was redacted for privacy.

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Signature was redacted for privacy.

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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 A¹ 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. Vitemin 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 vitemin. 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 main-The exact menner in which it functions during fat tenance. metabolism does not appear to be known. J. C. Drummond², who has worked on this, was not able to obtain any direct relationship between Vitemin 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 Mendel³ indicates that fats are dispensable constituents of the diet provided there is present a suitable source of Vitamin A.

The verious 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 vive. It may be that part

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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. Harden⁴ calls attention to an unaccountably increased activity of yeast juice which contains zymase, if again boiled yeast juice is added. In the same publication⁵ 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. Villaroel⁶ has observed that extracts of Vitamin B, obtained from yeast, panereas and from testes sugment 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. Sordelle⁷ that the extracts

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containing B are complex and that it is necessary to show that the stimulation is due to Vitamin B is justified. U. Sanimerteno⁸, 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 Yoshio⁹ 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.

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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 lipsse 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

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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. Hawk¹⁰ states that pancreatic lipsse 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. Na2HPO4. in the medium.

Preliminary experiments indicated that 0.5 gms. panereatin 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 Na₂HPO₄ 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

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of a separatory funnel and the last traces removed by means of anhydrous CaCl₂. 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 Buell¹¹. 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 4. 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

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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

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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:

Tolucl 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 CO2 free distilled water was used for effecting this transfer. 250 cc. of CO2 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 end the condenser was made with a Kjeldshl nitrogen bulb. All glassware was rinsed in warm CO₂ 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 H₂SO₄ being carried over into the distillate.

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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.

		;		ion Con	stitue	ints				Cc. of
Exp. No.	Flask No.	Gms. Pan- cree- tin	Gns.* Vita- min A.(BF) Sand Prep.	Gms. Na ₂ HP04 12 H ₂ 0	H20 CO2	Ethyl	Drops	Incu-	Temp	N/10 NaOH to neut. Batyr- ic Acid
19	0 1 2 3	0.500 π π π	0.000 0.500 1.000 1.500	81	5.00 n n	1.00 т п п	Approx.025 c.c. Not controlled.	41 11 11 11	25 ⁰ C. 7 7	6 29 8.44 9.03 10.39
28	0 1 2 3	9 म 11 11 11	0.00 0.500 1.000 1.500	п	2.00 ^π ^π	हा स 11 11	9 п п	41 11 11	30 ⁰ C	5.67 6.12 6.51 6.79
	: 0 : 1 : 2 : 3	е п п и и	0.000 1.500 1.000 1.500			0.40 11 11 11	17 12 12 12		स स ग ग	5.10 7.69 8.22 8.93

TABLE I

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

[©]Pancreatin, ether extracted for 12 hours.

#Solution of Na₂HPO₄ 12 H₂O. 1 cc. = 0.25 gms. Na₂HPO₄ 12 H₂O.

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.

13 0 F FIG. 11.00 Ехр. 19. 10.00 Stimulation of Lipase by a Vitamin A Concentrate 7.00 8.00 7.00 6.00 0.0 5 2:0 FIG.Z. NB BV Exp. 28. Stimulation of Lipase by a Vitamin A Concentrate 8.00 7.00 C.C. 01 6.00 5.00 0.0 2.0 1.0 1.5 0.5 F16:3. 10.00 9.00 Ехр. 29 0.00 Stimulation of Lipase by a Vitamin A Concentrale. 7.00 <u>5</u>00 5.00 0.0 0.5 Z.0 1.0 Gms. Vitamin A (B.F.) Sand Preparation

The acidity of the ethyl butyrete was determined in Exp. 29. 0.1 cc. of the stock ethyl butyrete 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 $4\frac{1}{2}$ 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 & Concentrates upon Lipase Activity.

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

	-		Rea	ction Co	nstitu	ints				Cc. of
	:		Gms.		:		:	;	: :	N/10
_		Pan-	:C.S.	:Cc.	:Cc. ;		:Tol-			NaOH to
	:Flask		:011	:Na2HPO4	:H20	Ethyl	: uol	:Incu-	:Temp::	neut.
No.		:tin	Sand_	:Sol	: (002 ;	Buty-	Drops:	betion:	: :	Butyr-
		in :	Prep.	•	:free);	rate	:	:	: :	ic Acid
	:	E.E.	*				•		; ;	
	:		;	;	*	;	:	*	: .	
	: 0	0.500	:0.000	5.00	5.00	0.50	: 6	: 8 <u>3</u>	30°C	7.49
34	-		:0.20		: n	: 1	: .	; n ²	ं ग	8.40
••			0.40	1	: 12	. 11	: 11	: 📅	n .	8.79
	: 3		0.60	1 1	; n ;	त	: 11	1 11	я <u>–</u>	8.55
	: :	;		• • • • • • • • • • • • • • • • • • •	:	:	:	:	; .	0100
		•	4	•	•	,	4	-	÷	
	: 0	0,500	:0.000	2.00	: 5.00	0.50	: 5	5 2/3	30°G	8.48
	: 1		0.150		1 N S	5 1	: 11	Ξ π ²	- 51	8.10
	2		·0.300		ः मु	n i	• n	ः स	, n	7.77
20	. 3		0.450		• 11	π	1 II	ំ ក	្តី អ	7.45
39	: 4	5 H	0.600		1	. R	1	1 11	- 17	8.17
	· 4 · 5	: 11	0.750		1 11	1 11	11	ः ग	n	7.88
	• 6	i 11	0.900		ះ រា	і п	រ ា	7 11	ग	8.40
	7	: n	1.050		; п	1 17	1	- n	11 11	7.70
	8	ः च	:1,200		. 17	π	: <u>n</u>	- n	n	7.82
	: 9	1 11	0.000		1 m	n	\$ ग	• n	<u>п</u>	6.75
	2	•	ā., ,.,	÷ .	;		:	*	 • *	

TABLE II

*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.

 $\frac{1}{7}$ 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 same. 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.

	:	; 	React	ion Cons	stitue	ats				Cc.
Exp. No.	Flask No.		Gms. Sea	Cc. Na2HPO4 Sol.	Cc. H2O (CO2	Cc.	T01-	:Incu- :bation;	Temp	N/10 NaOH to neut. Buty- ric
26	: : 0 : 1 : 2 : 3	11	0.00 0.50 1.00 1.50	11 12	2.00 n n	1.00 π π π	5 π π	42 n n n	30 ⁰ 0. " "	Acid 6.22 6.18 4.80 5.04
268	: : 0 : 1 : 2 : 3	л П	0.00 0.50 1.00 1.50	77 72	57 57 77 77 77	。 将 疗	77 77 77 77	11 77 77 17 17	17 77 32 35	9.32 9.65 9.60

TABLE	Ι	I	Ι
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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.

16 a FIG. 4 10.0 -xp. 34 9.0 The Effect of a Film of Oil on Lipase Activity 8.0 7.0 C.C. of N/10 No: OH. 6.0 F1G. 5 9.0 EXP. 39. 8.0 The Effect of a Film, of oil on Lipase Activity. 7.0 6.0 0.0 19 1.3 0.6 1.Z. Grams Sand Oil Preparation.

3. The Effect of Toluol on Lipase Activity in the

Presence of Vitamin A Concentrate.

	:	1	:	Read	stion Co	ostitu	ents		:		: :Cc.
	•		:		•				:		:N/10
	:	- 1		Vit. A	-	-	. Cc. :		:_ :	1	:NaOE
				(B.F.);			Ethyl			Temp	
0.	: N(). :	: tin ;		Na2HP04						;neut
	:	;	: :	Prep.	Sol.	free)	: rate;	Drops	:bation:	:	:Butj
	:		: :	: :		:	i		:	:	: ric
	:	:	: :		:	:	: :	:	: :	:	:Acid
	:							, ,	•		:
	:		; ;	; ;	:	:	:	;	;	•	:
	:		: :			:	: :		:	:	:
	-		:0.500:				: 0.50;		· •	30°C	
		2	: "	0.300	=	: ^म	1 ⁵⁷	4	ः ग	-	:15.2
40	: 3	3 :	: "	11	: n		: т :	6	: "	-	:14.
	: 4	1	: 17	11	11	: ग	: ¹¹	8	: 77	: ⁿ	:14.6
	; {	5	1 T 1	13	н н	: n	: ¹¹ 1	: 10	: 17	ग	:13.
	:		: :		:	:	: :	:	•	:	*
	:		:	: :	• ·		:		:	;	;

TABLE IV

NOBN

i The second ارده این کارم و رموه ایم ورد. از در ایم از این ایم ورد را در مرد این این اعماد مح n an an an an ann an a' an an Anny 1997. The standard of the formation of the formation of the second standard of the second standard st (1) A series was generated as a series as an experimental second of the series of արտարությունների համանակությունը համարությունը համարությունը համարությունը։ Մերջանությունը համարությունը համարությունը համարությունը համարությունը համարությունը համարությունը համարություն Այս համարությունը համարությունը համարությունը համարությունը համարությունը համարությունը համարությունը համարությ raya 116 dala barak mendakarat kasalangka ilekkin lehin lera kasil ak dari meta الارام المراقبة المر مراقبة المراقبة المرا The second se <u>in the second second</u> ter a state and the second 计 加拉 法推动执行 17.0 ու հետ հայտությունները։ Հայաստանությունները հայտապատճառում։ Դեն հայտների հայտանին հայտներին։ Դեն հայտների հետ հայտների հետ հայտների հետ հայտներին։ Դեն հայտների հայտների հետ հայտների հետ հայտներին հետ հայտներին։ n and a second 16.0 Several status applicants have in EXP. 40 a 15.0 The Effect of Toluol upon the Acceleration caused by a Vitamin A Concen-0 14.0 یا او رس بعرسی رسال از مرابعه ای مقدمت را international interesting 13.0 U 0.0 4.0 6.0 8.0 10.0 indaalla akis Drops of Toluol (preservative) a med an energy straight a dimension for a factor to the second state of the second state of the second state o A second state of the second sta and the property of the second sec د. المراجع من المراجع الم المراجع က ကြားသြားနဲ့ ကျက်သြားသည့် ကျက်သည်။ ကြိုက်ကြားနဲ့ ကျက်သည်။ ကြိုက်ကြားသည့် ကျက်သည်။ ကျက်သည်။ ကြိုက်သည်။ ուներագրում է հետև ուրը։ Դենքագրում է հետև ուրը։ وم از الأصح بي وي يوالية الي العالي . والا الا مراجع الا المحمد الما المراجع المراجع المراجع ال kale jule yere se apprennen er mer essere af der frå dale adle.

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 emount of toluol has some solvent action upon the Vitamin A concentrate, thereby withdrawing the Vitamin A from the seat of hydrolytic action. Altho the smount of toluol in Exp. 28 end 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 smount 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. Warden¹² 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,

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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 rether high titer is due in pert to some butyric soid performed by hydrolysis. Some butyric acid has no doubt been formed thru action of the medium used.

	•	:	5	Read	tion Co	nstit	vents				Cc. of
Exp.	::	Plask:	Pan- crea-	:011 :(CS) :Sand	Cc. NagHPC4 Sol.	:H20 :C02	:Buty-	uol	Hrs. Incu-	Temp	H/10
7401		T. A.				.1100	.1986			-	Acid
	*	0	0.000	0.000	3.00	:2.00	: :0.50cc.	: 4	8 <u>3</u>	30°C	2.72
41	•	1 2		0.300		ू स • ग	រ រ រ	л . п	77 . • 77 .	भ ग	2.65
		3	π	:0.900	П	т т	н Н	- 11	- 11 - 11	17	2.15
	•	4	ų.	:1.200	17		• • • • •	•	• •	• ³ •	: 1.58

TABLE V

19 a

i i dan seria. An dan seria ang seria An dan seria ang seria ് പ്രതിക്കാന് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത്. പ്രതിക്കാന് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത്. പ്രതിന്ത പ്രതിന്താന് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത് പ്രതിന്ത്. പ്രതിന്ത میسید. در سیاسی این از میشوند کرد میشند از آنها در معرف این میشوند که میشون و است. میشوند میشونم باشد از میشوند کرد میشوند در میشوند که میشون و م (a) An experimental and the second s Second seco 그는 것 그 같은 것을 다시는 것을 것은 것은 것은 것을 못 못 했다. £..... - Mar Constant (Constant) and a second in notice in the second of the second state of the second s المراجع مراجع المراجع ا -Analis ing M**uda**ta di kabupatèn kabupatèn م از ایند دهم مواجه ما F1G. 7. transfer a la fall présente construir é înspir set que nome trans ⊈lite died. e units con the same of the second (a) A set of the se MO BN OW 4.0 March March 199 Hold In 199 Exp. 41. 3.0 and a second يو الديني ويون The Effect of a Film of Oil on Sand as a Hydrolytic Agent. 2.0 المربعة المراجع 0 12 C line (142) (nampolitic provider grander dage de la merine en particular grander en la provider de la provider d 1. 0.0 転送する 74 37<u>2</u>2 448 755 a construction of the second sec i i se 0.3 0.6 1.8 1.2. Grams Sand + Film of Oil. Récent d'Are d'automa Roma hactories de la comp ter Maasaala sekarata er Martha, 1982er ber Market M. C. Market and M. S. Market an Market and M. S. Market and M. otnika obi usinsk si verstre dettjo rekse in razykny: Maadal doorweyeer the gaa The best for the second second the second s file and the same of the second s ÷ ...

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 sulphoric soid 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 smount of Vitamin A sand preparation, it might be argued that the increased entrainment of HgSO4 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 emount of volatile fatty acids in varying quantities of Vitamin A concentrate. In both experiments the procedure was identical in respect to the addition of sulpharic acid to liberate the butyric acid.

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TABLE VI

Exp. No.	Flask: No.	Gms. Pan- cres-	Gms. Vit. A BF.	Cc. Na ₂ HPO ₄ Sol.	:Co. :H20 :CO2	: Cc. Ethyl	Tol-	; bation	Temp.	Cc. of N/10 NaOH to Neut. Butyr- ic Acid
31	0 1 2 3	ग ग	0.000 0.500 1.000 1.500	17 17	buty.	dilute ricacia dilute lalcoho	ў 9 п П	10 11 11	30 ⁰ 0 " "	6.90 7.11 6.96 6.70
27		ਸ ਸ	0.000 0.500 1.000	л П	2.50 1 n 1 N 1 N		: 9 : п : п : п	4 17 11 11	21 21 22 27	0.20 0.21 0.29 0.21

*To each flash was added 0.5 gms. of Na2HPO4 12 H2O 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 COg 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:

Flask		: Observed • : Cc. of NsOH d : N 0.2117 :	: Actual :
7	1.00	2.95	: 3.04
2	1.00	2.50	2.99
3	1.00	2.95	2,95
4	: 1.00	3.13	ti das das tas tas
	*	4	:

TABLE VII

7. The Effect of Cholesterol upon Lipase Action.

Cholesterol and lecithin are common constituents of fats, especially those of animal origin. E. F. Terraine¹³ 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

: :Gms.:Cho-:Cc. :Cc.:Cc.: :Hrs.:Temp:NaOH t Exp.:Flask:Pan-:lest-:NagHPO4:H2O :Ethyl:Tol-:Incu-: :neut. No.: No.:crea-:erol:Sol. :CO2 :Buty-:uol :bation: :Butyr-		:		:	React	ion Cor	stitu	ents		:	Cc. of
No. : No. : crea-: erol : Sol. : CO ₂ : Buty-: uol : bation: : Butyr- : tin : Sand : : : : : : : : : : : : : : : : : : :	Exp.	: : :F	lask		Cho-	Cc.					
$\begin{array}{cccccccccccccccccccccccccccccccccccc$:crea-	erol : Sand :	S01.	:000	Buty-	: 001	bation;	Butyr- ic Acio
	.58	: : :	3 4	1 11 17 1 11	:0.000 :0.500 :1.000 :1.500	8.00 11 11	11 11 11	ा ग म	: 4	: R : R : R	" : 4.70 " : 4.98 : " : 4.30

8. The Effect of Sodium Oleste upon Lipese Activity.

Commercial oleic soid was treated with sodium hydroxide in such a manner that not all the oleic soid was neutralized. The resulting soap solution was allowed to dry after which it was extracted several times with other 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.

-23-

23 a الم المحمد المراجع المحمد a la parte en activitario de la composición de la composición de la composición de la composición de la composi in strates -FIG. 8 ې و مانې پر و و مانې Exp. 58. 6.0 The Effect of Cholester upon Lipase Activit 10 201 5.0 0 0 0 Ø 49 a series and a series of the series of th A series of the 7.0 an de la provienció Se de la provienció 0 20 ť ť لىكى والمكركة المالية من المراكة المقالم أواليكا. الما معرفة المال العلم من المعاصف الرائي المناه منها 1.0 nas dipa Theallense in preparity large series and provide by the subgroup spec 1.0 on an alpha alo a second albe 2.000 parage ond a 3.04 See 1 122 िहास सम्बद्ध Grams Cholesterol Sand Preparation. مدر والشرية الولو والإنشاء والروائل مار الارتباط المراجع المحمد الارتباط والارتباط n an an ann an 151. Tarr a' gcantairte ىلىم ئىلىمىنى ئۇيۇنىڭ ئايار. بالا ئايەر قارى تەر قار and a second second 1.1 fer see of tracticate to the state of the term that the set is the second second second second second second s n an de service and a service and the service of the service service and the service serv n fan 1997 - Engelska skriger og samme 1993 af skriger og sam i som skriger fan fan skriger fan s ار مع میں ملاقات ا مار اس محمد بقاری ا i san i sa nang desiding de lieur prodes See State مان باللہ المرام کار کار اف راحمہ الحکو میں جو

ጥል	BLE	IX
	التدامنياس	مشعد

	;		:				tion Con	ne	stitu	16	nts			;	4 4 1		Cc. of
Exp. No.	: : :1	flask No.	Pe CI	18. 10- 168-		3	: Ce. Na ₂ HPO ₄ :Sol.	4:	COo	:	Cc. Ethyl Buty-	. 0	201- ;	Hrs. Inco bati	-	Temp	N/10 NaOH t neut. Butyr-
	*	· ,	:ti :		Sai Pre		*	:	free	:	rate	:	TODA				ic Aci
	:	1	:	.500	:0.0	200	: 3.00	:	2.00	:	0.50		4	. 9		30°Q	13.51
	•	2			:0.			:	- n	:	11	:	ਜ :			- π	12.77
		3	:		:0.1				11	:	11	:	π ;	: 11	,		13.57
	•	4	•		:0-			1	17	:	77		17	11		. n - i	16.55
43	-	5	:		:0.4			:	स	:	11 -	2	H 2	11	1	1 11	15.10
		6	1		:0.1				TT	:	77	:	11	· 11	1	: **	16.90
		7	:		:0.0			1	n	;	11	:	n i	स स		1 11	16.89
	-	8	-		:0.			-	n		£2	:	n	п		1 11	16.61
	;	9	:		:0.8			:	T	:	27	:	17 - 5	11		• n .	18.41
	;	10	:		:0.			;	53	:	71		11	17		i n	17.90
	•		:		•		:	;		:		:			1		

Reference to the acceleration by sodium cleate 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 other 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 other extract before this was washed with distilled water. The final wash water gave no precipitate or cloudiness when tested with AgN03. 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-

24 a an an ann an Staine a Tha an Staine an Stain للغ أدر المعرام مست میں بڑے ہے۔ بیٹ یہ دیکی بڑی i in line t trans an de ina na Talina ----- Lots and Findersense and a set of second seco and a state of the second s 7.5 and a second and the second and a second s FIG. 10 الهام من الدينة (1977) - المحمد المراجع مراجع المراجع ا بالوصحيفة بالمصافقان الغير المدارك الأكرامين. الأيار المدينة معهم المراز 19.0 DEIN 180: 0 Exp. 43. The Effect of Socium Oleate upon Lipase Activity. 17.0 160 0 23 15.0 じ じ 14.0 ا من المركز معني المركز من الم المركز المركز معني محكميتها المركز من الم 13.0 TOR NA CONTRA and the second s 12.0 0.0 0.2. 0.4 0.6 0.8 1.0 and a second second والمتحدث فتنا Grams Na Oleate Sand Preparation. $\frac{1}{2} = \frac{1}{2} \left(\frac{1}{2} + \frac{1$ fritation in the safet -------983.5

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 Lipsse 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.

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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.		:	Hrs. Incubation	Cc. of N/10 NaCH Required to Neut. Butyric Acid
49	Butter	1 2 3 4 5 6 7	0.000 1.000 0.450 0.900 1.350 1.800 2.150	; 11 ; 11 ; 11 ; 11	6 5/6 11 11 11 11 11 11	8.89 7.74 8.69 8.51 8.84 7.98 8.69
51	Cod Liver Oil	5 6 7 8	0.000 0.450 0.900 1.350 1.800 0.000 0.450 0.900 1.350 1.800	ग ग ग ग ग ग	41 17 11 11 11 11 11 11	$\begin{array}{r} 4.20 \\ 4.69 \\ 4.75 \\ 4.97 \\ 5.02 \\ \#0.25 \\ 0.29 \\ 0.24 \\ \hline 0.33 \end{array}$
66	Palm Oil	1 2 3 4 5 6 7 8 9 10	0.000 0.500 1.000 1.500 2.000 2.500 3.000 3.500 4.000 0.000	; 17 ; 17 ; 17 ; 17 ; 17 ; 17 ; 17	6 n n n n n n n n	4.00 5.85 4.88 6.14 5.73 5.35 4.53 5.74 3.42

*Standard reaction constituents, 0.5 gms. pancreatin, 3 cc. Na2HPO4 solution, 2 cc. of H2O CO2 free distilled, 0.5 cc. ______thyl 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.		Hrs. Incubation	Cc. of N/10 NaOH Required to Neut. Butyric Acid
	Wheat Embryo	5 :	0.000 0.500 1.000 2.000 2.500 3.000 3.500 0.000	ग ग ग ग ग रा रा	5 π π π π π π π	4.21 4.68 5.10 4.92 4.68 5.03 6.49 5.50 3.29
39	Cotton Seed 011	: 3 : : 4 : : 5 : : 6 : : 7 :	0.000 0.150 0.300 0.450 0.600 0.750 0.90 1.05 1.20 0.00	17 77 77 77 71 71	5 2/3 17 17 17 17 17 17	8.48 8.10 7.77 7.45 8.17 7.88 8.40 7.70 7.82 6.75
69	Glyce- rol	: 1 : 2 : 3 : 4 : 5 : 6 : 7 : 8 : 9 : 9	0.000 0.500 1.000 2.000 2.500 3.000 3.500 0.000	त 11 11 11 11 11 11 11 11 11		3.60 3.47 4.00 3.43 3.90 4.35 5.43 4.80 3.00

-28-

TABLE X (Cont'd)

Exp.		Flask No.	Gns. Fat or Oil Sand Prep.			Cc. N/10 NaOH Required to Neut. Butyric Acid
68	Nujol	1 2 3 4 5 6 7 8 9	0.000 0.500 1.000 1.500 2.000 2.500 3.000 3.500 0.000	11 11 11 11 11 11	9 11 11 11 11 11 11 11 11	3.98 3.21 5.50 5.10 5.55 5.10 5.77 5.68 4.57
65	: : :0live : 0il : :		0.000 0.500 1.000 1.500 2.000 2.500 3.000 0.000	17 17 17 17 17 17	67 11 11 11 11 11 11 11 11 11 11 11 11 11	5.00 5.18 4.52 5.69 4.91 5.44 4.75 4.18

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29a FIG. 5 9.0 Exp. 39. 8.0 The Effect of a Film of Cottonseed Oil on Lipase Activity. 7.0 6.0 0.0 1.3 1.0 1.5 2.0 FIG. 12 60 5.0 0 0 0 40 Exp. 67. 0.70 The Effect of a Film of Wheat Embryo Oil on Lipase Ectivity. 0.0 0.5 1.0 1.5 2.0 FIG. 15 6.0 400 3.0 Exp. 68 The Effect of a film of Nujol on Lipase Activity 0.0 0.5 1.0 10 13 30 95 1.5 Grams Vitamin A Vehicle

296 Franc FIG. FI The Effect of a Film of Butter Fat upon Lipase Activity 9.la 8.0 7.0 6.0 1.0 1.0 25 0.5 1. 7.0 FIG. 12 Exp. 51 The Effect of a Film of Cod Liver Oil upon Lipase Activity. 60 5.0 5 4.0 5 3.0 1.0 0.5 1. 1.0 2.0 F1G.13 6.0 ð 5.0 4.0 EXP. 66. 3.0 The Effect of Palm Dil upo FaF. 7.0 0.0 0.5 3.5 1.0 1.3 Grams Vitamin A Vehicle

290 FIG. 14 Exp. 69. The Effect of Glycerol on Lipase Activity. 6.0 5.0 40 Ø 0 3.00 يو محموليون (ماده مو 2.0 cc of N/10 NAOH. 0.0 30 FIG. 16 65 The Effect of a Film of Olive Oil on Lipase Activity. 6.0 1222 500 40 1 ÷ 3.0 0.0 0.3 Grams Vitamin A Vehicle Sand Preparation

PA	BLE	77*

Exp. No.	Fat or Oil	Flask No.		:	Hrs. Incubation	Cc. N/10 NaOH to Neut. Butyric Acid
	:	-	 }	F +		}
1	:	1 4	.0.000		: 4쿨 :	2.40
1	:	2 :	0.500	: n	: 17 :	2.90
78	Palm :	12345	: 1.000	• • • • • •	. . .	2.85
:	: 011 :	4	1.500	: 17	: 7	4.55
:	: :	5	2.000	11	: 17	2.89
:	:	:		;	:	
	:		,	•	•	
	: :	6 1	0.000		. म	2.41
:		7 3	0.500	: <u>!</u>	। ग	2.59
79	Nujel:	8 2	1.000	: 11	- ਸ	2.81
	: :	9 :	1.500	; 1	т	: 3.20
:	:	10	2.000		: स	3.29
:	:	:		:	•	•
,	;			:	b	•
:		1 :	0.000		: 5	2.33
	Wheat :	2 3	0.500		र ा	: 3.60
80 3	Embryo:	3	1,000		: R	3.65
1		4	: 1.500	1	: 17	3.00
	: :			*	:	:

*Standard reaction constituents.

If the study had been confined to the action of cottonseed oil, butter fat and cod liver oil upon lipsse 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 Figsind 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 lipsse and a stimulatory action on the part of the vitamin

30 a F16.17 ار المراجع الم المراجع EXD. 78 The Depressing Acti 40 of Toluol upon the Acceleration of Lipase caused 3.0 by a Film of Palm Oil. G 20 الهارية موجوا بها بالارتباع براعما المحطمين بالبوكر متراكر تتحجر بالتهم والإستيكية الالمامة /.0 1910 -0.0 the build of a first of FIG 18 for sample of the second state of the (1) The second s Second s Second sec second sec 4.0 to Exp. 78 .; 3.0 Similar Filmis nujól.) يور بر بر . در اند د الالكاني معدد والمحاصر الالكاني محمد والحاصر المحاط القرو ò 2.0 1.0 0.0-nga Manang Kabupatèn K (1) A second s second secon Ū المارية مريح الأربين. تحق المريح الإستنقاق وتاليون میں برا ہو ہے۔ اورانیسی کر مکرور ہوت -----geographic in the case were gui in in in Sin in in Sin in in FIG. 19 ار این شیمه موجود مدین از این این این این این این ا Exp. 80 ارس المراجع المحمد المراجع الم المراجع في المراجع الم and a straight and a 2.2.2 Similar to Exp. 78 ; (Film is wheat embroit) *4.0* ار بیتر از این المروزی ایر ایک این المید تدریدی 3.0 1315.2 2.0 nietz st (1) A set of the se 1.0== en al estimation at 0.0----2.0 1.0 1.5 2.5 0.0 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 embry's oil do not confirm this assumption. In Figures 12 and 15 it is shown that these substances accelerate the activity of lipsse. 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

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

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.

Source of Vitamin A	Gns. Fat or Oil Saponified	: Gms. Extract
Cod liver oil	300	2,9942
Lard	: 300	1.6904
Cottonseed cil Inactivated	300	2.0446
butter fat	260	2.1457
Alfa <u>l</u> fa	* * · · · · ·	8.9616

Table XII

*2578 gms. alfalfa extracted with 95% C2H50H.

-32-

TABLE XIII*

	:	Če	of N/10 Butyric	NaOH to ne 3 Acid	ut.
Type Vitam Pre	in A :	Oil Vit. A	Cotton- seed oil Vit. A Prep.	Vit. A Prep.	Insct. B.F. Vit. A Prep.
Exp	. No.	55	: <u>54</u>	56	53
Incub	ation :	4	: 4 :	4	; 4
Flask No.	: Gms. : : Sand : : Prep.:				; ; ;
2	: 0.000 : 0.500 ; 1.000 : 1.500 : 2.000	4.06 5.00 5.15	3.92 3.90 5.31 4.81 5.95	3.03 3.80 5.09 4.20 3.98	5.41 4.90 4.80 4.20 4.18
Exp	. No.	63	59	61	60
Incub	ation :	9강	101 101	10	: <u>5</u>
Flask No.	: Gms. : : Sand : : Prep.:				:
1 2 3 4 5 6 7 8 9 10	0.00 0.50 1.00 1.50 2.00 0.00 2.50 3.00 3.50 0.00	7.00 6.06 6.50 6.50 5.42 6.20	4.87 7.08 6.06 6.08 4.45 6.85 6.48 5.73 4.22	7.40	4.52 6.98 4.57 5.90 6.10 4.02 4.79 5.10 6.33 3.19

*Standard reaction constituents used.

33 a 77 FIG. 20 EXP. 55 6.0 The Effect of VitaminA Concentrate made from Cod Liver Oil upon Lipase Activity 5.0 4.0 3.0 Exp. 54 FIG.21 6.0 The Effect of Vitamin A Concentrate made from Cottonseed Oil upon Lipas Activity. 5.0 6.0 FIG. 22 Exp. 56 The Effect of Vitamin A Concentrate made from Lard upon Lipase Achivity A. 0 30 Exp. 57 6.0 The Effect of Vitamin 5.0 Concentrate made from Inactivated Butter Fat 40 upon Lipase Activity 3.0 0.0 2.5 0.5 1.5 2.0 1.0 Vitamin A Sand Prenaration Grama

33-6- \mathbf{Y} FIG. 24 12.2 7.0 المراقبة الأسرابية أن المراقب المراقبة في المراقبة المراقبة المراقبة المراقبة المراقبة المراقبة المراقبة والمر مراقبة المراقبة المرا مراقبة المراقبة المر 6.0 anna agus an tuair tuair Tuair agus an tuair Tuair agus an tuair XP 63 5.0 -ا الما ماني أن الارد أن أن أن أن المراجع الماني التي يت الماني من المراجع من المراجع الماني الماني الماني الم المان الماني من المانية من من المانية المراجع المانية ومنا من المراجع من المراجع من المراجع الماني المانية الم اف محد أواد التي حريف الأربي بالاد والتكان. ديسجوج بين دارد بعالم براجي مي معيام والعون. 4.0° an sea ann an 1910 ann an san Albana. Na chuir dha ann ann an san Albana. jiri jiri si sani. FIG.25 1941 - 14 - 14 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 -1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 1971 - 197 Construction of the Manager Manager ا داده الجامع المراجع منه ومنها و المراجع المراجع المراجع المراجع المراجع المراجع المراجع المراجع المراجع المر المراجع Marganian and a sign of the Marganian strategy of the second system of the Marganian strategy of the Margania 7.0 6.0 EXP. 59 5.0 يې مې د د مې مې د کې مې د د مې مې د د د د 4.0 مراجع المراجعية المراجع من مراجع من المراجع مربعة أسار المراجع المراجع المراجع بشبية أأت £..... FIG. 26. in the second 9.0 *0*.0 Exp. 61 7.0 6.0 8 م م المراجعة ال محمد المحمد المراجعة ا 5.0 1 ş. 0.32 FIG. L.T. and a second sec - 5 د ماد ها المعروب الدامي مراجع الم التي المراجع EXD.60 6.0 É. F. 5.0 Experiments Showing the Effect of VitaminA 4.0 Concentrates upon Lipase Activity Fig. 24 from Cod Liver Oil Fig. 26 from Lard 3.0 0 Inactivated " Cotton Seed Oil 25 27 Butter Fat. 0.0 0.5 1.0 2.0 25 Grams Vitamin A Concentrate

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 lipsse 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 slfalfs 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

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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 elfalfa upon lipase activity. This concentrate was made according to the directions of Steinbock and Boutwell¹⁴. Approximately 2500 grams of dry powdered alfalfa meal of good quality was twice extracted with cold 95 percent elcohol. The alcohol extract was concentrated by distilling off the elcohol. 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-

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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.

Flask No.	: Gms. : Vit. A Send 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
	•	•

TABLE XIV*

*Standard reaction constituents, 4 drops toluol. Incubation period 72 hours.

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and the second - Comparing Any Sector Control and Secto Sector Control and Sector الدارية والمراجعة بالمراجبة والمراجعة والمراجعة والمنطقة المحمطة والمراجعة والمحرفة المراجع محمد المراجع المراجع gia, áras spelespigg pill egyperet lais is i de liter de la service de la service de la service de la service d la iya şiya iya (a) A second se second sec ار د همید که برای د معرد سردند. است همهای ارد برای استان از از مراد د Encode a second s Second se Second s Second se a the second pression of the second المعرور می رودم از ا این مراجع از مراجع در مراجع از مراجع از ا FIG. 28 8.0 Exp. 48. The Effect of a Vitamin & Concentrate from Alfalfa upon Lipase Activity. 7.0 6.0 الم محمد المحمد الم المحمد ا این میکنون دست ایک باید این ا این از ماهاردها از ایک باید ایک باید 5.00 الملك، ما ملاح المراجع الجنوب ملاحة التيام التحادية (الملاحيات المالية أن أن المراجع التي . الاحاد الحاد المراجع المراجع المراجع المراجع المراجع التي المراجع التي المراجع المراجع المراجع المراجع المراجع الما الملك الذي المراجع 4.0 Here Market and the second second ان المراجع من المراجع (a) and an and a second sec المحمد المعرفين المحمد التركيم المعرفين المحمد المعرفين المحمد المحمد المحمد المحمد المحمد المحمد المحمد المحم المحمد يەر دەرمىيو بەر بىرە . بەر مەروبار بېرىدى بەر مەروبار بېرىدى 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 Grams Vitamin A (Alfalfa) Sand Prep. un sete spij og set eksel state in el forste fin الم محمد المحمد الم محمد محمد المحمد الم Note: This concentrate shown by animal growth to be deficient in Vitamin A Para and an angle and a second

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

The acceleration of lipsse 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 snimal 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 dist 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.

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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.

Concentrate		Co. per 1000 gms. Ration	•		
Cottonseed oil Inactivated	: 7/7/25:	16.5	: :0.3374		 •
butter fat Lard			:0.4119 :0.2788		 •
Cod liver oil Alfalfa	7/7/25 7/7/25	16.5		8/6/25 8/6/25	:0.3374 :0.100

TABLE XV

*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 Buell¹⁴ 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

	•		میں کوریٹر اف میٹ افروری ویشار ا							_
Vitamin A : Vehicle	í •	But	ter Fa	at		Co	ttons	eed O	<u>il</u>	
Rat No.	21	22 :	23	: : :	:: 25 ::	26 :	27 :	28 :	29	
Sex	9	₽:	ô :	ô :	ô	ô	₽:	ç :	ę	-
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:				94:	95;:	•	79:	81:		
Date 6/19;		82:		85;	101:		80:	97:	75	
of $6/29$			96:	87:	105::		82: 77:	100:	76 _70	
Weigh- 7/7 : ing 7/14:				-		#104*		*101:		
7/26					141:		*90:	#90:	200	
8/5	· · · · · ·			109:	167;		877:			
8/18	-			133:	186:		:	:		
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20. • • • • • • • • • • • • • • • • • • •	······································				1	• •				
Vitamin A	:		•	•.*		:		,		
Vehicle		Cod L	iver	011 ·	:		Ler	.d¢		
Ret No.	30	31 :	32 :	33	34		36	37 :	38:	39
Ser	ô	₽;	ô	ç:	ô	*	ð	ę:	₽.	Q
- / /	: :	:	:	:	:	•			;	
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- 1-	: 56:									
6/5	: 72:	80:	74:	70:	64:	-	66:	60:	69	87
6/5 6/13	: 72: : 88:	80: 93:	74: 80:	70: 86:	64: 69:	: 74 :	66: 73:	60: 65:	69. 75.	87 88
6/5 6/13 Date 6/19	: 72: : 88: : 98:	80: 93: 96:	74: 80: 99:	70: 86: 93:	64: 69: 72:	: 74 : : 86 :	66: 73: 76:	60: 65: 76:	69 75 75	87 88 93
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6/5 6/13 Date 6/19 of 6/29 Weigh-7/7	: 72: : 88: : 98: : 99: : 139:	80: 93: 96: 104: 121:	74: 80: 99: 113: 144:	70: 86: 93: 103: 126:	64: 69: 72: 85: 123: 143:	74 : 86 : 86 : 70 :	66 73 76 66 57 &	60: 65: 76: 66: 60:	69: 75: 75: 76: 72: *76:	87 88 93 90 *84 *84
6/5 6/13 Date 6/19 of 6/29	: 72: : 88: : 98: : 99: : 99: : 139: : 158:	80: 93: 96: 104: 121: 127:	74: 80: 99: 113: 144: 156:	70: 86: 93: 103: 126: 129:	64: 69: 72: 85: 123: 143:	74 : 86 : 86 : 70 :	66 73 76 66 57 &	60: 65: 76: 66: 60:	69: 75: 75: 76: 72: *76: 73:	87 88 93 90 *84 *84 *81
6/5 6/13 Date 6/19 of 6/29 Weigh- 7/7 ing 7/14 7/28	: 72: : 88: : 98: : 99: : 139: : 158: : 193: : 193:	80: 93: 96: 104: 121: 127: 143:	74: 80: 99: 113: 144: 156: 179:	70: 86: 93: 103: 126: 129: 173:	64: 69: 72: 85: 123: 143: 176:	74 86 86 70 1070 11 17/20 11 17/28	66 73 76 66 57 &	60 65 76 66 60	69: 75: 75: 76: 72: *76:	87 88 93 90 *84 *84
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6/5 6/13 Date 6/19 of 6/29 Weigh- 7/7 ing 7/14 7/28	: 72: : 88: : 98; : 99: : 139: : 158: : 193: : 215:	80: 93: 96: 104: 121: 127: 143: ; 163:	74: 80: 99: 113: 144: 156: 179: 194:	70: 86: 93: 103: 126: 129: 173: ; 150:	64: 69: 72: 85: 123: 143: 143: 176: : 190:	74 86 86 70 (7/20) (7/28)	66 73 76 66 57 &	60 65 76 66 60	69: 75: 75: 76: 72: *76: 73:	87 88 93 90 *84 *84 *81

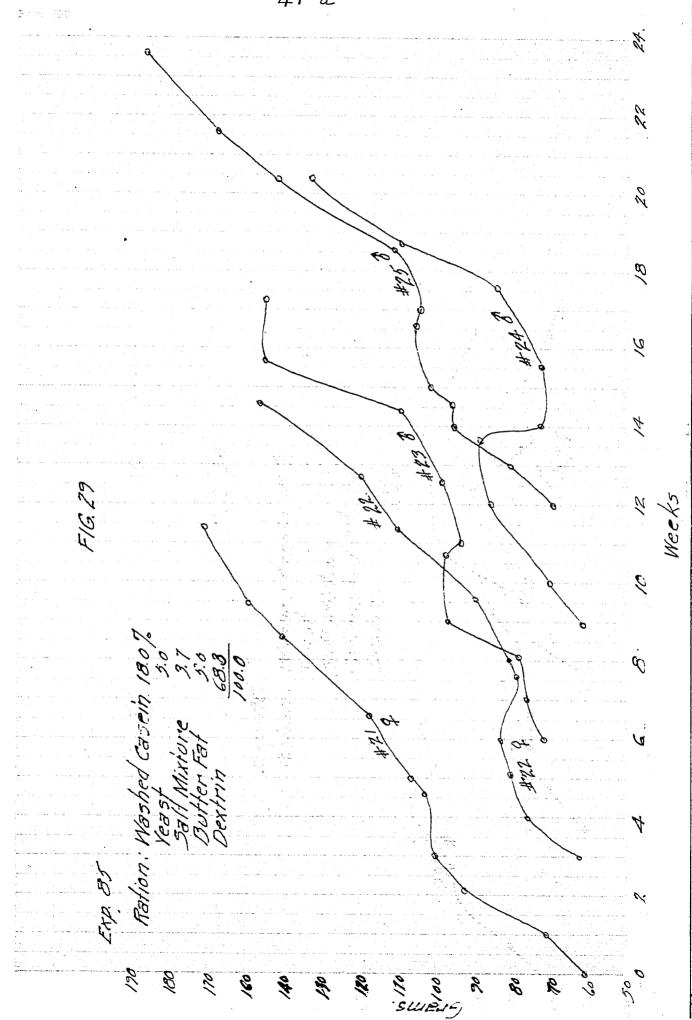
%Killed, ear infection. %Heavy line indicates addition of an amount of concentrate equivalent to 5% of the original source of the concentrate.

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Data	6/13: 6/19:	83	• •	77 80	•	78 78	;	68	:	70 81	10			90	:	92	÷	00 92	;	98
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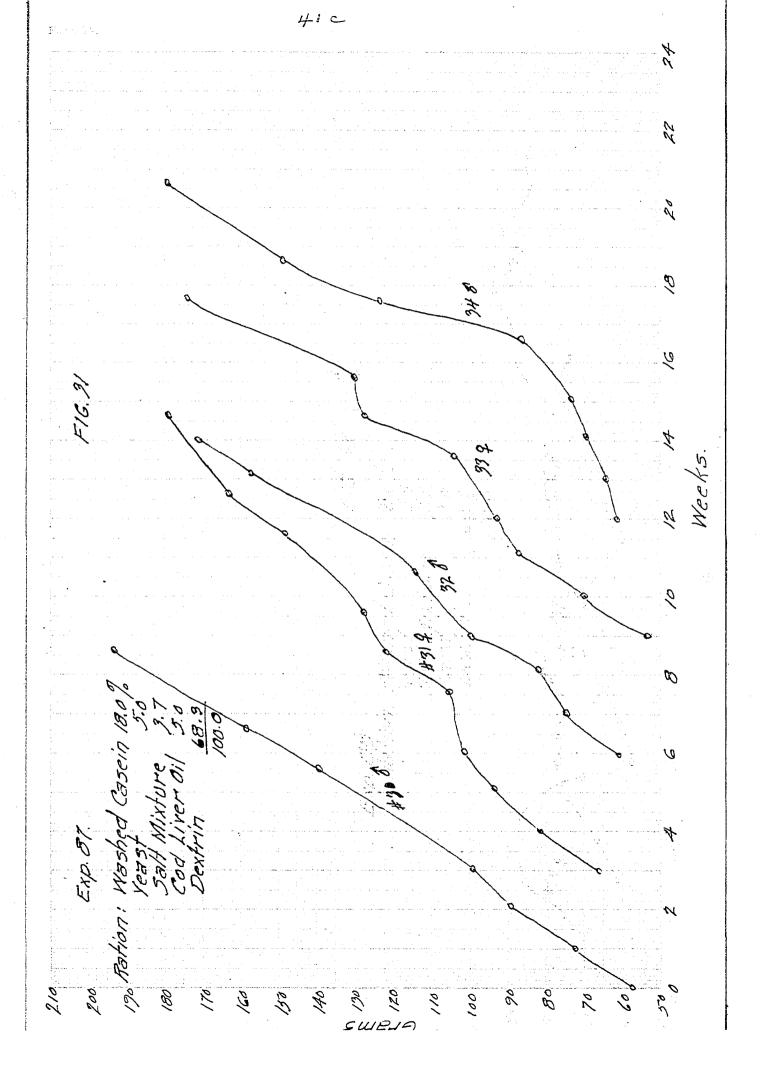
*Eyes sore.

@ Killed.

Found dead. # 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.



41 a



41 d $\mathcal{D} \in \{1, \dots, n\}$ 64. 22 20 #39 4 9 9 20 4 F16. CASCIN 18.0% 0.00 Weeks. N 10 185/10 1/10 Ŋ \mathcal{O} 20 0/ N 0 100 00 00 2 00 20

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419 $\mathbb{P} \otimes \mathbb{I}$ 24 22 20 Q #159 D 2 1 7/7/25 by 16 5 c.c. ether solution concentrate made from cod liver o. Supplemented 3/6/25 by BR 5 cc of Same Solution F16. 33 Weck 17 Ō 10:0 2.00 7/1// Washed Casein Yeast Salt Mixture 0 0 lemented NO NO Ю EXD. 03. N 0 20 150 130 120 20 *C* 60 H(00 20 WP

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	7/14 7/20 7/28 8/5	144 143 147 140		78# : : :	86 : 87 : 62# : :	96 99 99 92

TABLE XVI (Cont'd)

*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 Na₂HPO₄ 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 scap solutions by means of ether. J. Lewkowitsch¹⁵ states that palmitic sciā has thus been extracted from a sodium palmitate solution by toluene. 0.0319 grams palmitic acid (Kahlbaum) was dissolved in 95% sthyl 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 CO2 free distilled wa-There remained a residue after removing the ether by ter. 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 scaps 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(NO₂)₆. The resulting product accelerated lipase activity decidedly. Hence it seems that the acceleration is

-43-

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

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

TABLE XVII*

*Standard reaction constituents, 2 drops toluol. Incubation period 52 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 al¹⁶ 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-

44a ه این هایه د وسیده چر بردمههای سازی د الى ئەلەر بەلەر سەرە ئەلەر بەلەر بەلەر مرد المرد العربية مراجعة المراجع المراج والمراجع مراجع المراجع and a state of the second s England en and the second and the second sec te a statut para la seconda de la second المراجع میں ہے۔ اور ایر دیکر میں جانب الدارية ممارية الروارية المعرفة المعرفة والرواد والمعرفة معمر والمارية المعرفة والمعرفة والمعرفة والم and and the second s Second and the second se free and the second second FIG. 38 The Effect of Vilamin A XD. 64. concentrate (free from 趋行 fatty acids) upon Lipase العين المراجع والمناطرة المعجرات مراطيني 7.0 a di di Jaca da n n angelik Nitika nag 6.0 5.0 4.0~ الله اليوليم الموضوف أحمد التاريخ والمار. در الإرجام التاريخ الماري التاريخ التاريخ ا المواج معروم که به وصورهای این اور دارد. امواج معروم که به وصورهای این اور دارد این مواج وارد. امواج و این این این این اور و این این در دارد این دارد. 3.0 Second and constrained at the second s 2.0 0.0 1.5 - 2.0 1.0 25 3.0 0.5 n a ann anna 1999. Ann - A Ann - A 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 scaps and crystalline in character. This product was used in testing lipse activity.

A direct relationship between the enimal 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 scaps 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.

-45-

	e of Vit. A nd Prep.	:Cod Liver : Oil	: Cotton- Seed Oil		Inactivated Butter Fat
Ex	p. No.	: 74	: 77	76	75
Flask: No. :	Gms. Sand Prep.	: : Cc. o :	: f N/10 Nec :)H	
1 2 3 4 :	0.000 0.500 1.000 1.500	: 2.86 : 3.25 : 3.40 : 3.30 :	2.55 3.60 3.60	3.50 4.12	

*Reaction Constituents:

0.5 gms. pancreatin

0.5 cc. ethyl butyrate

5.0 cc. H₂O distilled CO₂ free

5.0 cc. Na2HPO4 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 Vitemin 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 fet 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 cleate 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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