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Title

A MATHEMATICAL THEORY OF VITAMIN A METABOLISM

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MANUSCRIPT REPORTS of the Biological Stations

57	Cooke, N.E.	A Mathematical Theory of Vitamin A Metabolism.
264	Battle, H. I.	Food of the Hake on the Atlantic Coast.
365	Andrews, C.W.	Report of Pollett River Smolt Trap Operation 1947.
366	Andrews, C.W.	Report on Salmon Scale Readings, Pollett River.
369	McKenzie, R.A.	(1) Smelt Tagging, 1943-44, Miramichi Area. (2) Smelt Movements, 1943-44 tagging, Miramichi Bay. (3) Smelt Movements, 1944-45 Tagging, Miramichi Bay. (4) Smelt Tagging in the Miramichi Region, 1944-45.
378	Medcof, J. C.	1944 Investigations Oysters and Clams.

A MATHEMATICAL THEORY OF VITAMIN A METABOLISM

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ABSTRACT

Several hypotheses of the mechanism of storage and utilization of vitamin A are developed from fundamental postulates. These hypotheses are compared with experimental data obtained from the brill (Eopsetta jordani). Although enough experimental data are not available to prove statistically the mechanism for this fish, the most probable result is obtained from the hypothesis which is also the most probable from a priori reasons. From the data at hand the size of a further experiment is outlined which will determine the true relationship with a probability of 0.95.

INTRODUCTION

Ever since the discovery that vitamin A was stored in the liver of animals, investigations have been carried out to determine the mechanism whereby the vitamin A was stored there and eventually used. Some of the more recent important investigations in this field have been carried out by Lemley, Brown, Bird and Emmett, (1947), Caldwell, Macleod, and Sherman (1945), Campbell, Udiljak, Varmolinsky and Sherman (1945) and Rohrer and Sherman (1943). Some earlier investigations were made by Sherman and Storms (1925), Sherman and Boynton (1925) and Sherman and Cammack (1926).

The fact that some fish livers are so very potent in vitamin A has been a source of wonderment for many workers and considerable investigation has been done on the vitamin A content of fish livers. The question is: Is a fish capable of synthesizing vitamin A in its organism or does it only ingest vitamin A or a closely related provitamin A in its food? has puzzled the minds of workers in this field. Both hypotheses have been supported by more or less indirect proof. The case for ingestion alone has been upheld by Macpherson (1937), Drummond, Zilva and Coward (1922) and Jameson, Drummond and Coward (1922). Drummond, Zilva and Coward state the case for ingestion in the remark, "There appear to be no adequate grounds for doubting that the ultimate origin of the vitamin A in the marine oils and liver oils is represented by the marine plants possessing photocatalytic pigments..."

On the other hand, the case for synthesis is presented by Morton (1940) who, although he states, "Animals have the power of converting certain carotenoids into vitamin A, but there is no evidence that vertebrates can synthesize either carotene or vitamin A de novo", says, in reference to the work of Wald and Zussman (1937) who showed a close relationship between astaxanthin and vitamin A, "The structure (of astaxanthin) does not lead to the supposition that astaxanthin is a provitamin A but Wald's findings indicate a close connexion with vitamin A and if it turns out that fishes possess a peculiar power of utilizing the pigment, it might be possible to account for the huge reserves of vitamin present in so many fishes, because they consume vast quantities of minute crustaceans, the predominant pigments of which belong to the astaxanthin series". This view is echoed by Rosenberg

(1942, pp. 73-74) who also points out that the mechanism for the accumulation of vitamin A in fish livers is certainly different from that of mammals or birds because of the occurrence of vitamin A_2 in fish livers. This is not the case in land animals which only get vitamin A_2 by direct ingestion from fish. (Edisbury, Morton, Simpkins and Lovern 1938; Lovern and Morton 1939; Lovern, Morton and Ireland 1939).

While this paper does not prove or disprove any theory so far presented, it is hoped that it will point the way for further experimental work which will answer the problem.

DISCUSSION OF THE DATA AND CONSTRUCTION OF THE EXPERIMENTAL CURVE

Recently Swain and Barraclough (1946) and Barraclough and Swain (1947) published data showing, among other things, that the total vitamin A in the liver of the brill (Eopsetta jordani) increased as the weight of the fish increased. The increase was slow at first but as the fish matured it became more rapid. Figure 1 shows this relationship (more will be said later about the data used in plotting figure 1). Other workers had also noticed the same relationship with other fish. The most important researches in this line were made by Ripley and Bolomey (1946) on the soupfin shark (Galcorhenus zyopterus), Lovern, Edisbury and Morton (1933) on the halibut (Hippoglussus hippoglossus), Pugsley (1939a) on the Pacific halibut (Hippoglossus stenolepis), Pugsley (1939b) on the grey cod (Gadus macrocephalus), Pugsley (1939c) on the dogfish (Squalus sucklii), Pugsley, Morrell and Kelly (1945) on the Atlantic cod (Gadus morrhua), Swain and Barraclough (1947)

on the Long jaw flounder (Atheresthes stomias), and Edisbury, Lovern and Morton (1937) on the eels (Anguilla vulgaris and A aucklandi).

Although most of the above mentioned authors seem to have realized that some fundamental reason existed for this variation, most of them were satisfied merely to report this relationship and no one presented a satisfactory explanation. Perhaps the basic change, i.e. the continual increase in the total vitamin A content which takes place in the system, was obscured by the purely seasonal one, i.e. the fluctuation of the oil content of the liver, which of course causes the potency of the oil to vary seasonally, too. Lovern, Edisbury and Morton (1933) tried to link this seasonal change to some change in the nature of the diet or eating habits of the fish, but, since they could not find any definite relationship, they satisfied themselves with "tentative explanations".

That the change in the total vitamin A with increasing size of the fish is the more fundamental change and also that the total vitamin content does not fluctuate seasonally but increases steadily is shown by several papers. McKercher (1946) followed the seasonal variation of the oil content and the vitamin A potency of the livers of three species of flat fish; the brill, the rock sole (Lepidopsetta bilineata) and the lemon sole (Parophrys vetalus). Barraclough and Swain (1947) determined the vitamin and the oil content of two sets of brill caught at the extremes of the fluctuation; one set in October when the oil content was high and the potency low and the other set in April when the converse was true. The very significant fact which they

concluded is in their words, "They (the data presented) show that the actual amount of vitamin A in the brill livers did not vary with the seasons since there was as much present in April-caught fish as in October-caught fish of the same length". This same fact was also shown for entirely unrelated fish, i.e. the soupfin shark by Ripley and Bolomey (1946) and the Atlantic cod by Pugsley, Morrell, and Kelly (1945).

All the data that have been used are set forth in the first two columns of table I. To avoid any variations due to sexual differences only female fish were considered. It might have been better to have considered only male fish but the necessary data were not available. Any variations because of different locality were kept to a minimum since all the fish were caught in the same area off Port San Juan on the west coast of Vancouver island.

The method used to obtain a smooth curve which approximates the most probable one can be described best by reference to table I. The first column contains the values of the weight in ounces from 0 to 64. The second column contains the raw data. As is seen, for certain values of w there is no value of R, and for other values of w there is more than one value of R. The third column contains data adjusted from those of column 2. If only one value appears in column 2 it is merely reproduced in column 3. If two or more values occur in the second column the adjusted value is an average. If no value occurs in the second column then the adjusted value was obtained by interpolating graphically from an approximate curve drawn through the original data. The average values are marked with a super "a" and the graphical

values with a super "g". The fourth column, headed graduated data, was calculated by the Whittaker-Henderson graduation method. The particular scheme used was that described by Macaulay (1931). The values from 61 to 64 in the adjusted data column were necessary for the proper calculation of the last column. If these values are not included the third differences of the points needed for the start of the second half of the calculation become negative and the curve begins to open downward. The cause for this phenomenon is the fact that one of the highest values is at w = 57 and the following values are slightly lower. Since it is obvious that the curve keeps opening upward the extra data obtained by graphical extrapolation are necessary to assist in obtaining a valid approximation to the true relationship. These extra adjusted data mean, of course, that the graduated data were calculated beyond the point w = 60. These extra values are not shown, however, because they are only aids in the calculation and have no physical significance. It is unfortunate that this rather cumbersone procedure had to be used. With sufficient data as outlined in the Discussion below much simpler methods involving groups of data could be used.

TABLE I

R = Vitamin A in the liver in USP Units x 10^{-5}			W = weight of fish in ounces	R = Vitamin A in the liver in USP Units x 10^{-5}		
Data	Adjusted data	Graduated data		Data	Adjusted data	Graduated data
-	extrapolated by means of constant 2nd differences $D^2=0.0013$	0.000	33	-	4.50[g]	5.419
-		0.047	34	2.14;3.98	3.06[a]	5.880
-		0.094	35	2.76;16.32	9.54[a]	6.314
-		0.143	36	5.25;18.85	12.05[a]	6.675
-		0.193	37	4.74	4.74	6.957
-		0.245	38	-	6.70[g]	7.204
-		0.297	39	-	7.25[g]	7.469
-		0.351	40	2.04;6.07		
-		0.407		8.36	5.49[a]	7.804
-		0.463	41	-	8.25[g]	8.241
-		0.521	42	-	8.75[g]	8.779
-		0.580	43	-	9.50[g]	9.402
-		0.641	44	6.76;11.50		
.73	.73	0.703		11.76;18.30	12.08[a]	10.079
-	.705	0.766	45	7.54	7.54	10.780
.68	.68	0.842	46	10.21;16.97	13.59[a]	11.484
1.10	1.10	0.938	47	-	12.00[g]	12.167
.89	.89	1.057	48	6.89;12.62		
1.60	1.60	1.203		22.76	13.92[a]	12.824
1.00;1.32	1.16[a]	1.378	49	-	13.50[g]	13.471
1.17;1.22			50	-	14.10[g]	14.147
1.33;1.70	1.36[a]	1.582	51	21.17	21.17	14.906
.94;2.17	1.56[a]	1.815	52	6.00;6.16	6.08[a]	15.808
1.07;1.27			53	-	16.40[g]	16.917
4.27	2.20[a]	2.069	54	12.91;39.17	26.04[a]	18.231
-	2.20[g]	2.335	55	7.41	7.41	19.712
3.65	3.65	2.605	56	-	19.00[g]	21.312
1.44;3.86	2.65[a]	2.873	57	37.90	37.90	22.929
2.02	2.02	3.140	58	-	21.20[g]	24.481
3.01	3.01	3.409	59	28.34	28.34	25.989
2.77;7.69	5.23[a]	3.679	60	20.27	20.27	27.528
4.86;7.05	5.96[a]	3.952	61	-	28.50[g]	
2.67	2.67	4.245	62	-	31.50[g]	
2.57;4.10			63	-	33.50[g]	
4.56	3.74[a]	4.582	64	-	36.00[g]	
-	4.25[g]	4.977				

MATHEMATICAL THEORY

There are several mechanisms whereby the accumulation of reserve of vitamin A in fish's liver may occur. By postulating these mechanisms, by developing relationships from these postulates in terms of the weight of the fish and the amount of vitamin A in the liver, and then by comparing the derived relationships with those found by experiments, an hypothesis can be constructed which can be checked against further experimental work.

Probably the best approach to this problem is to consider the overall aspect of some substance, call it compound X for lack of a better name, which occurs in the fish's food and is eventually converted to vitamin A and stored in the liver. Compound X may be vitamin A itself, a provitamin A like carotene (Neilands 1947, Morton and Creed 1939), a carotenoid such as astaxanthin which is not considered a provitamin A (Morton 1940), or some entirely unrelated compound, such as starch.

Figure 2 represents the mechanism diagrammatically.

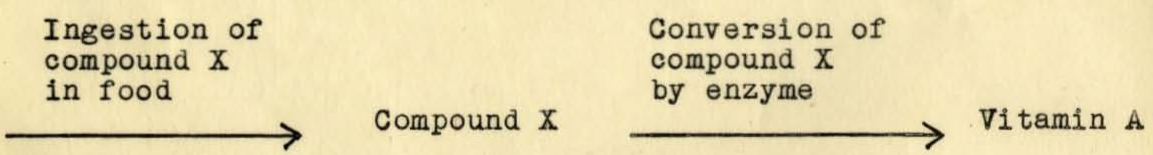

Figure 2

It was first thought that this enzyme system occurred in the liver itself (Moore, 1931, Olcott and McCann 1931) but now it has been shown that the intestinal wall is the true site (Wiese, Mehl and Deuel 1947; Mattson Mehl and Deuel 1947; Glover, Goodwin and Morton 1947; Ahmad 1931).

That the liver is not the only storage place for vitamin A in fish has been shown by Lovern, Edisbury and Morton (1937) and Edisbury, Morton, Simpkins and Lovern (1938). It is unfortunate that the data available were not on the total vitamin A in the fish, but it is probable that what can be said in regard to vitamin A storage in the liver could also be said with minor modifications in regard to the total vitamin A in the fish.

In this system there are two rates which might act as the "master" rate and control the whole system. If the rate of ingestion is slower than the rate of conversion it will, of course, be the controlling one. This would probably be the case if compound X were vitamin A itself. On the other hand, if compound X occurred in large amounts in the food, the rate of conversion would be the slower. It would be quite possible, of course, for two compound X's to exist and both mechanisms to exist side by side.

Consider the over-all material balance of vitamin A in the liver. Let R be the residue found there, S be the total amount stored, and U be the total amount utilized, then

$$R = S-U \qquad (1).$$

If the rate of ingestion is the controlling one, we may consider that the amount of compound X ingested is proportional to the amount of food ingested; this will vary directly with the size of the fish and also at the rate at which the fish is growing. By taking the units of time to be years, any seasonal changes (such as changes in the eating habits of the fish, the seasonal variation in compound X, and so forth) will be averaged and of no

consequence in this discussion.

Expressing the above postulate mathematically, we have

$$di/dt = k_1 w + k_2 \, dw/dt \qquad (2)$$

Where i is the total amount of food ingested

t is the time

w is the weight of the fish

di/dt is the rate of ingestion

dw/dt is the rate of growth

If the rate of conversion is the controlling one, it would seem plausible to assume that this rate would be proportional to the size of the organ in which the conversion is taking place which, in turn, would be proportional to the size of the fish.

Mathematically this becomes

$$ds/dt = k_3 w \qquad (3)$$

where s = the total amount of vitamin A made and ds/dt = the rate of synthesis.

The utilization of vitamin A by the animal organism has been the subject of considerable research. The minimum requirement has been shown for all species of mammals investigated to be proportional to the body weight. Guilbert and Hart (1935) showed this for cattle, Guilbert, Miller and Hughes (1937) for sheep and swine, Irving and Richards (1939) for rats, Lewis, Bodansky, Falk and McGuire (1942) for rats, Goss and Guilbert (1939) for rats, Phillips and Bohstedt (1938) for rabbits and Guilbert, Howell and Hart (1940) for horses. The work of Guilbert and his co-workers places the requirements for mammals at 4-6 μg of vitamin A per kilogram of body weight per day. The vitamin A requirements of fish seem to be lower than this value as shown by Tunison, Brockway, Maxwell, Dorr and McCay (1942), but

the assumption that the daily requirement in fish is proportional to the body weight appears to be a valid one. Expressing this relationship mathematically, we have

$$dr/dt = k_4 w, \quad \text{_____} (4)$$

where r is the total requirement and dr/dt is the requirement with respect to time or the daily requirement.

Although it has been quite well shown that the minimum requirements of vitamin A are as given above, evidence has also accumulated that the animal organism utilizes the vitamin A reserves in the liver in direct proportion to the amount of vitamin stored there. This was noticed by Baumann, Riising and Steenbock (1934) for rats, Davies and Moore (1935) for rats, Brenner, Hessler, Brooks and Roberts (1942) for rats, and Frey and Jensen (1946, 1947) for cattle. If this is the case it can be expressed mathematically as

$$du/dt = k_5 R, \quad \text{_____} (5)$$

Where u is the total amount used and du/dt is the rate of utilization.

To derive the desired relationship between the reserve in the liver and the weight of the fish, it is also necessary to know the relationship between the age and the size of the fish. This is given by the well-known autocatalytic equation (Robertson 1923 page 6).

$$dw/dt = k_6 w(a-w), \quad \text{_____} (6)$$

There are six possible cases which arise from the foregoing equations. They will be discussed in order of the complexity of the equation.

Case I

The storage comes from ingestion only, and the utilization is the same as the requirements. The following postulates are necessary:

$$R = S-U \qquad (1)$$

$$di/dt = k_1 w + k_2\, dw/dt \qquad (2)$$

$$dr/dt = k_4 w \qquad (4)$$

$$dw/dt = k_6 w(a-w) \qquad (6)$$

Equation 1 becomes

$$\frac{dR}{dt} = \frac{dS}{dt} = \frac{dU}{dt}$$

By definition, i = S and r = U

$$\therefore \frac{dR}{dt} = k_1 w + k_2 \frac{dw}{dt} - k_4 w$$

$$dR = (k_1 - k_4)w\,dt + k_2\,dw$$

From (6) $dt = \dfrac{dw}{k_6 w(a-w)}$

$$\therefore dR = \frac{k_1-k_4}{k_6} \frac{dw}{a-w} + k_2\,dw$$

Integrating,

$$R = -\frac{k_1 - k_4}{k_6} \operatorname{Log}_e(a-w) + k_2 w + C \qquad (I)$$

which is the desired relationship for case I.

Case II

The storage comes from synthesis only and the utilization is the same as the requirements. The following postulates are necessary:

$$R = S-U \qquad (1)$$

$$ds/dt = k_3 w \qquad (3)$$

$$dr/dt = k_4 w \qquad (4)$$

$$dw/dt = k_6 w(a-w) \qquad (6)$$

Equation 1 becomes

$$\frac{dR}{dt} = \frac{dS}{dt} - \frac{dU}{dt}$$

By definition,

$$s = S \quad \text{and} \quad r = U$$

$$\frac{dR}{dt} = k_3 w - k_4 w$$

$$dR = (k_3 - k_4) w dt$$

From (6) $$dt = \frac{dw}{k_6 w(a-w)}$$

$$\therefore dR = \frac{k_3 - k_4}{k_6} \frac{dw}{(a-w)}$$

Integrating,

$$R = - \frac{k_3 - k_4}{k_6} \text{Log}_e (a-w) + C \qquad \text{(II)}$$

which is the desired relationship for case II

Case III

Both mechanisms for storage exist simultaneously and the utilization is equal to the requirements. The postulates needed are:

$$R = S-U \qquad (1)$$

$$di/dt = k_1 w + k_2 dw/dt \qquad (2)$$

$$ds/dt = k_3 w \qquad (3)$$

$$dr/dt = k_4 w \qquad (4)$$

$$dw/dt = k_6 w(a-w) \qquad (6)$$

Equation 1 becomes

$$\frac{dR}{dt} = \frac{dS}{dt} - \frac{dU}{dt}$$

By definition,

$$i + s = S, \; r = U$$

$$\therefore \frac{dR}{dt} = k_1 w + k_2 \frac{dw}{dt} + k_3 w - k_4 w$$

$$dR = (k_1 + k_3 - k_4) w \, dt + k_2 dw.$$

From (6)

$$dt = \frac{dw}{k_6 w(a-w)}$$

$$dR = \frac{k_1 + k_3 - k_4}{k_6} \frac{dw}{(a-w)} + k_2 dw$$

Integrating,

$$R = -\left(\frac{k_1 + k_3 - k_4}{k_6}\right) \text{Log}_e(a-w) + k_2 w + C \quad \text{(III)}$$

which is the desired relationship for case III.

Case IV

The storage comes from ingestion only and the utilization is proportional to the reserve present.

The postulates necessary are:

$$R = S - U \quad (1)$$

$$\frac{di}{dt} = k_1 w + k_2 \frac{dw}{dt} \quad (2)$$

$$\frac{du}{dt} = k_5 R \quad (5)$$

$$\frac{dw}{dt} = k_6 w(a-w) \quad (6)$$

Equation 1 becomes

$$\frac{dR}{dt} = \frac{dS}{dt} - \frac{dU}{dt}$$

By definition,

$$i = S, \; u = U$$

$$\therefore \frac{dR}{dt} = k_1 w + k_2 \frac{dw}{dt} - k_5 R$$

$$dR = k_1 w \, dt + k_2 dw - k_5 R \, dt$$

From (6)

$$dt = \frac{dw}{k_6 w(a-w)}$$

$$\therefore dR = \frac{k_1}{k_6} \frac{dw}{(a-w)} + k_2 dw - \frac{k_5}{k_6 w} \frac{R dw}{(a-w)}$$

$$\frac{dR}{dw} + \frac{k_5}{k_6} \frac{R}{w(a-w)} = \frac{k_1}{k_6(a-w)} + k_2 \quad \text{(IVa)}$$

Since this differential equation is of the form $\frac{dy}{dw} + f(x)y = g(x)$ reduction to quadratures is accomplished by means of the well known formula

$$y = e^{-\int f(x)dx}\left\{\int g(x)\, e^{\int f(x)dx}\, dx + C\right\}$$

$$\therefore R = e^{-\frac{k_5}{k_6}\int \frac{dw}{w(a-w)}}\left\{\int\left(\frac{k_1}{k_6(a-w)}\right) e^{\frac{k_5}{k_6}\int \frac{dw}{w(a-w)}}\, dw + C\right\}$$

$$\therefore R = \left(\frac{a-w}{w}\right)^{\frac{k_5}{k_6 a}}\left\{C + \frac{k_1}{k_6}\int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}+1}}\, dw + \int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}}}\, dw\right\} \quad \text{(IV)}$$

which is the desired relationship for case IV. The integrals cannot be evaluated in terms of known functions for non-rational exponents. As will be seen later this is no serious handicap.

Case V

The storage comes from synthesis only and the utilization is proportional to the reserve present.

The postulates necessary are:

$R = S - U$ (1)

$\frac{ds}{dt} = k_3 w$ (3)

$\frac{du}{dt} = k_5 R$ (5)

$\frac{dw}{dt} = k_6 w(a-w)$ (6)

Equation 1 becomes

$$\frac{dR}{dt} = \frac{dS}{dt} - \frac{dU}{dt}$$

By definition,

$$a = S,\ u = U$$

$$\therefore \frac{dR}{dt} = k_3 w - k_5 R$$

$$dR = (k_3 w - k_5 R)dt$$

From (6) $dt = \frac{dw}{k_6(a-w)}$

$$\therefore \frac{dR}{dw} = \frac{k_3 w - k_5 R}{k_6 w(a-w)}$$

$$\therefore \frac{dR}{dw} + \frac{k_5 R}{k_6 w(a-w)} = \frac{k_3}{k_6(a-w)} \qquad \text{(Va)}$$

This equation is of the same form as equation (IVa) and, therefore, can be solved in the same way. It becomes

$$R = \left(\frac{a-w}{w}\right)^{\frac{k_5}{k_6 a}} \left\{ C + \frac{k_3}{k_6} \int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}+1}} dw \right\} \qquad \text{(V)}$$

which is the desired relationship for case V.

Case VI

Both mechanisms for storage exist simultaneously and the utilization is proportional to the reserve present.

The postulates necessary are:

$$R = S - U \qquad (1)$$

$$\frac{di}{dt} = k_1 w + k_2 \frac{dw}{dt} \qquad (2)$$

$$\frac{ds}{dt} = k_3 w \qquad (3)$$

$$\frac{du}{dt} = k_5 R \qquad (5)$$

$$\frac{dw}{dt} = k_6 w(a-w) \qquad (6)$$

Equation 1 becomes

$$\frac{dR}{dt} = \frac{dS}{dt} - \frac{dU}{dt}$$

By definition,

$$i + s = S, \quad u = U$$

$$\therefore \frac{dR}{dt} = k_1 w + k_2 \frac{dw}{dt} + k_3 w - k_5 R$$

$$dR = (k_1 + k_3) w\, dt + k_2\, dw - k_5\, R dt$$

From (6) $dt = \frac{dw}{k_6 w(a-w)}$

$$\therefore \quad \frac{dR}{dw} + \frac{k_5 R}{k_6 w(a-w)} = \frac{k_1 + k_3}{k_6(a-w)} + k_2 \qquad \text{(VIa)}$$

As before

$$R = \left(\frac{a-w}{w}\right)^{\frac{k_5}{k_6 a}} \left\{ C + \frac{k_1 + k_3}{k_6} \int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}} + 1} dw + k_2 \int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}}} dw \right\} \qquad \text{(VI)}$$

which is the desired relationship for case VI.

Determinations of the Constants of the Equations

Now, after having developed six different possibilities for the mechanism of vitamin A metabolism, it is desirable to determine the constants by the method of least squares and see how well the equations fit the data and also to see if the constants so determined are compatible in sign and order of magnitude with those given in the postulates. From the data of Swain and Barraclough (1946) and Barraclough and Swain (1947) a growth curve was constructed which had the equation:-

$$\text{Log} \frac{W}{61-W} = -0.3902(t - 6.1)$$

or $$\text{Log}_e \frac{W}{61-W} = 0.8984(t - 6.1)$$

Undoubtedly, these constants would be changed by a small amount if more data were available to determine them.

In order to lessen the computation involved in determining the constants of the equations, only every fifth value as given in the graduated column of table I was used. This device, while lessening the work by about four fifths, did not materially change the accuracy of the results. Since when the weight of the fish is zero the amount of vitamin A in the liver is also zero, the constants of all the equations were adjusted to fulfill this condition. Although

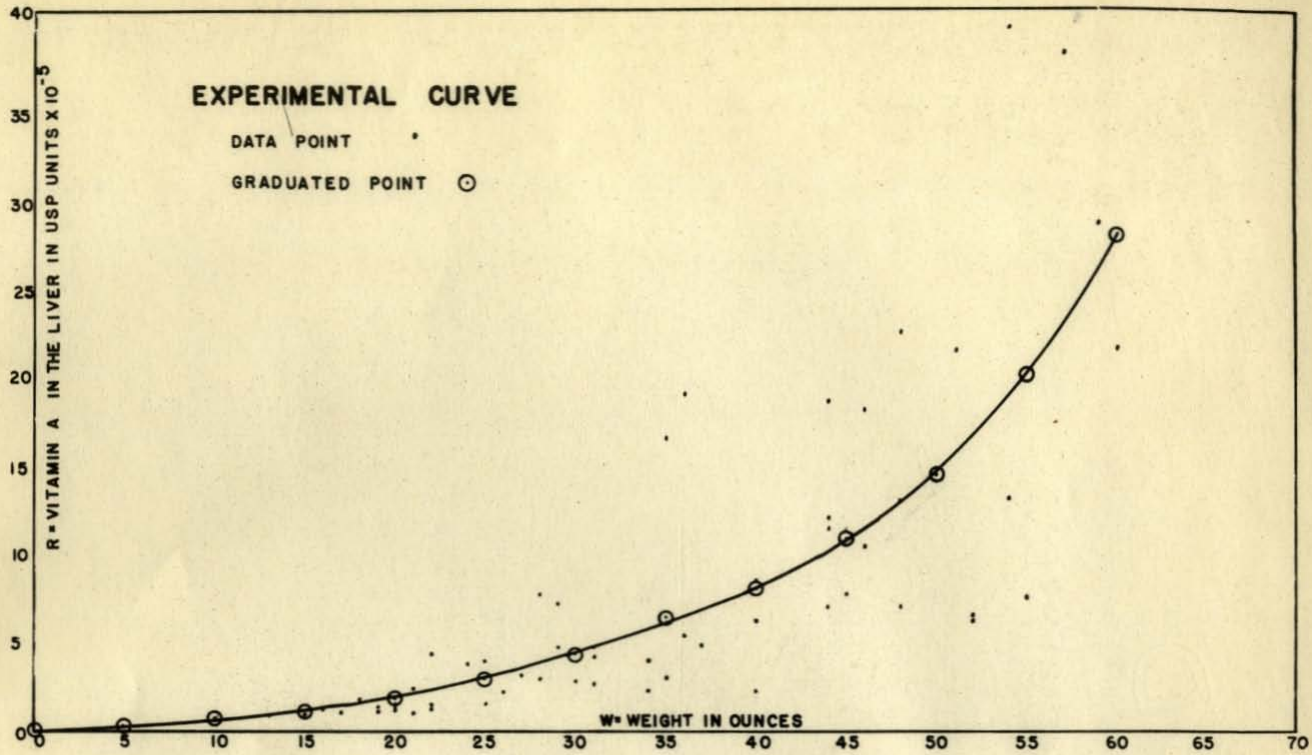

FIGURE I

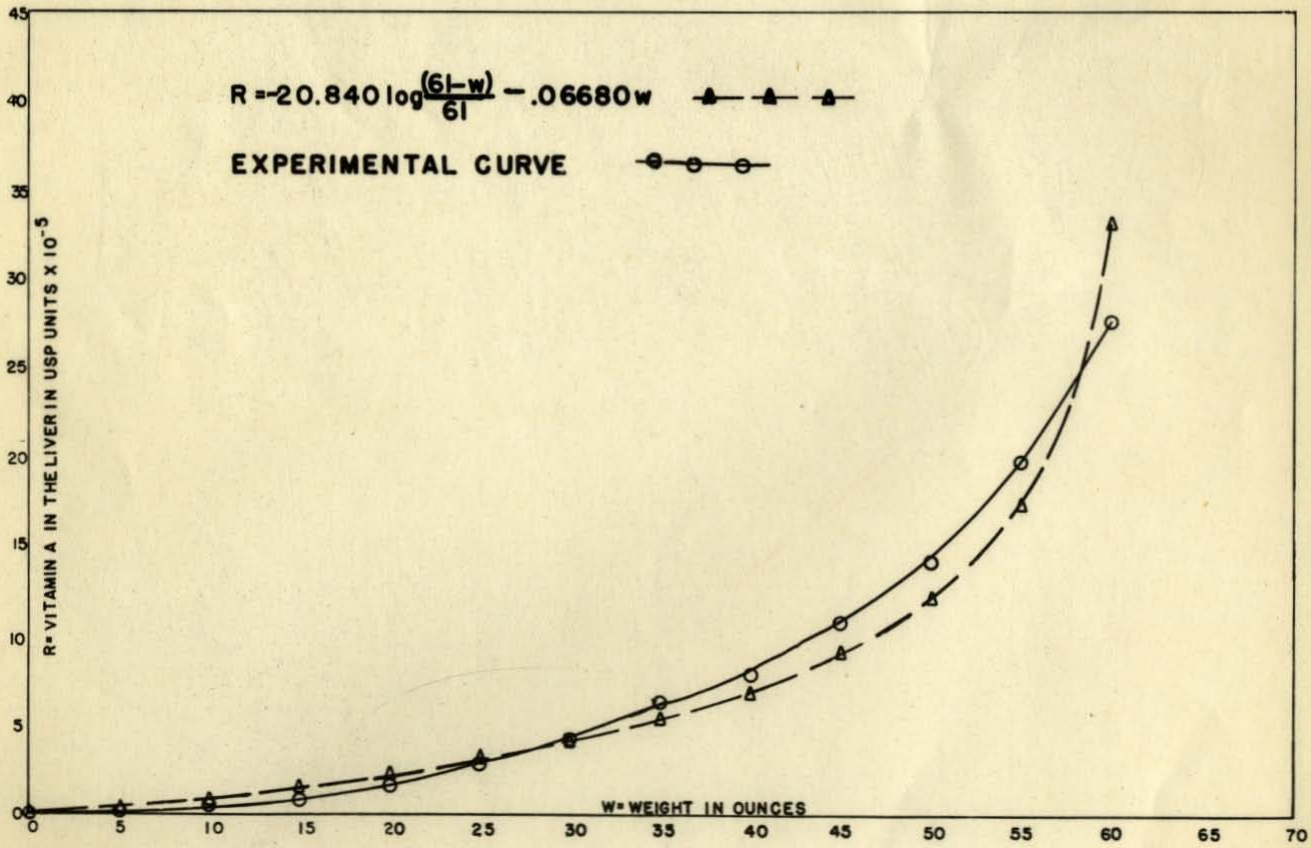

FIGURE 3

it might appear at first glance that the slope of the curve at the origin should be zero, there is no reason why it should be and consequently this restriction was not placed on the constants.

The equation for Case I is:

$$R = -\frac{k_1 - k_4}{k_6} \text{Log}_e (61-w) + k_2 W + C$$

or, since it passes through the origin,

$$R = -\frac{k_1 - k_4}{k_6} \text{Log}_e \frac{(61-w)}{61} + k_2 W.$$

Solving for the constants of this equation by the method of least squares it becomes

$$R = -20.840 \text{ Log } \frac{(61-w)}{61} - 0.06680 W$$

or

$$R = -9.0508 \text{ Log}_e \frac{(61-w)}{61} - 0.06680 W$$

This equation is plotted in figure 3 as is the experimental curve. The sum of the squares of the differences between the points on the equation and the corresponding points on the experimental curve is 47.4.

The equation for Case II is:

$$R = -\frac{k_3 - k_4}{k_6} \text{Log}_e (a - w) + C.$$

Similarly, passing the curve through the origin, it becomes

$$R = -\frac{k_3 - k_4}{k_6} \text{Log}_e \frac{(61-w)}{61}$$

Solving for the constants as before

$$R = -16.413 \text{ Log } \frac{(61-w)}{61}$$

or

$$R = -7.127 \text{ Log}_e \frac{(61-w)}{61}$$

This equation is plotted in figure 4 as is the experimental curve. The sum of the squares of the differences between the points on the equation and those on the experimental curve is 23.45.

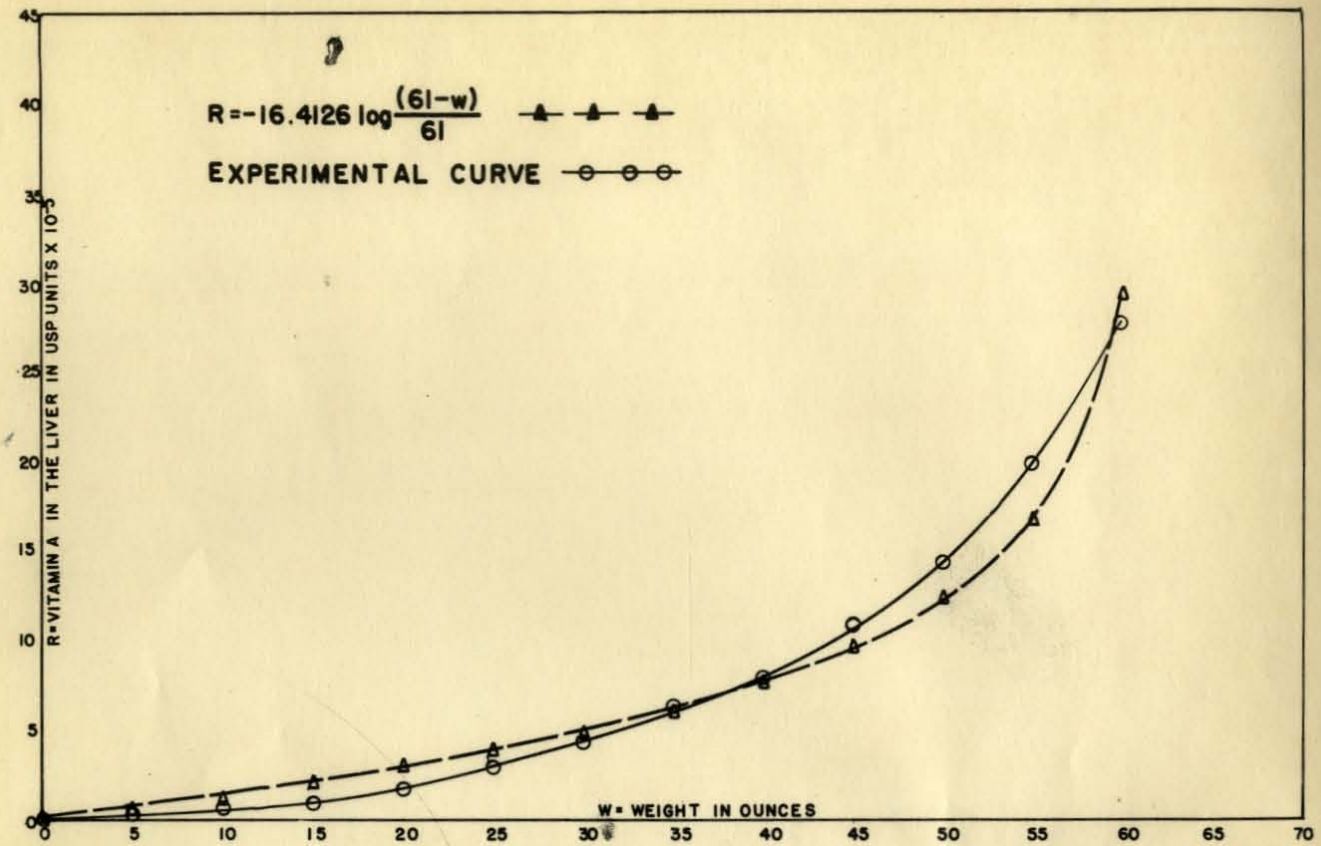

FIGURE 4

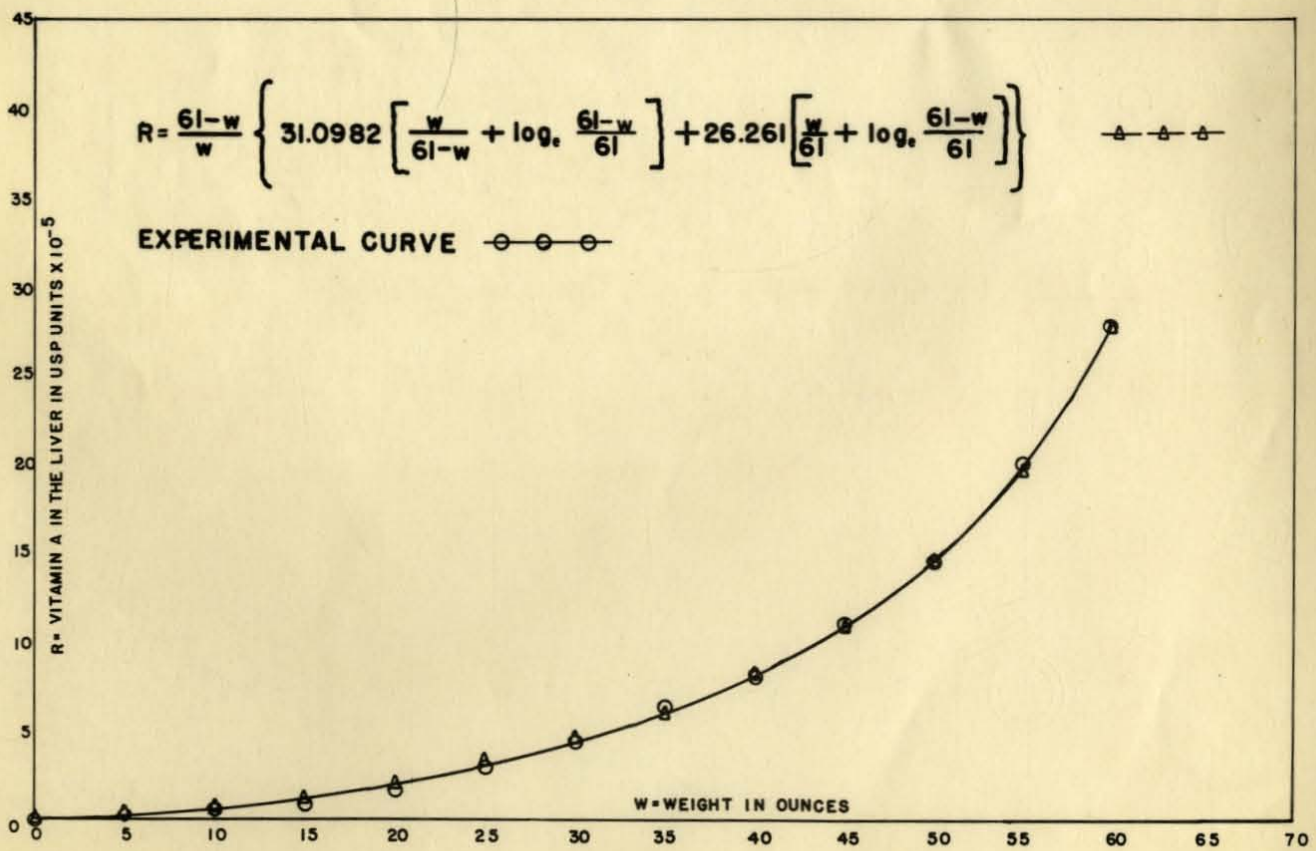

FIGURE 5

The equation for Case III is:

$$R = -\frac{k_1 + k_3 - k_4}{k_6} \operatorname{Log}_e (a-w) + k_2 W + C$$

which for purposes of least-square-fitting is identical with the equation for Case I. The constants are, therefore, the same and the equation is

$$R = -9.0508 \operatorname{Log}_e \frac{61-w}{61} - 0.06680 W$$

The equation for Case IV is:

$$R = \left(\frac{a-w}{w}\right)^{\frac{k_5}{k_6 a}} \left\{ C + \frac{k_1}{k_6} \int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}+1}} dw + k_2 \int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}}} dw \right\}$$

This equation as it stands is not integrable and is not linear in the constants to be solved for. To simplify the equation, let $v = \frac{w}{a}$ $\frac{k_5}{k_6 a} = n$; $\frac{k_1}{k_6} = P$ and $k_2 a = q$.

It then becomes

$$R = \left(\frac{1-v}{v}\right)^n \left\{ C + p \int \frac{v^n}{(1-v)^{n+1}} dv + q \int \frac{v^n}{(1-v)^n} dv \right\}$$

Now, assuming that $n = 1$ and integrating we have,

$$R = \left(\frac{1-v}{v}\right) \left\{ C + p \left(\frac{1}{1-v} + \operatorname{Log}_e(1-v)\right) + q \left[1-v-\operatorname{Log}_e(1-v)\right] \right\}$$

Now, since when $R = 0$, $v = 0$ we have

$$C = -(p + q)$$

and

$$R = \frac{1-v}{v} \left\{ p \left[\frac{v}{1-v} + \operatorname{Log}_e(1-v)\right] - q \left[v + \operatorname{Log}_e(1-v)\right] \right\}$$

Solving for p and q by the method of least squares, the equation becomes

$$R = \left(\frac{1-v}{v}\right)\left\{31.0982\left[\frac{v}{1-v} + \text{Log}_e(1-v)\right] + 26.261\left[v + \text{Log}_e(1-v)\right]\right\}$$

which, when changed to the variable w, becomes

$$R = \frac{61-w}{w}\left\{31.0982\left[\frac{w}{61-w} + \text{Log}_e\frac{61-w}{61}\right] + 26.261\left[\frac{w}{61} + \text{Log}_e\frac{61-w}{61}\right]\right\}$$

This equation gives an amazingly close fit to the experimental curve with the difference in the sum of the squares being only 0.9237. The equation is plotted in figure 5. Because of the very close agreement it was felt that any attempt to get a better value of n would be fruitless.

The equation for Case V is:

$$R = \left(\frac{a-w}{w}\right)^{\frac{k_5}{k_6 a}}\left\{C + \frac{k_3}{k_6}\int \frac{w^{\frac{k_5}{k_6 a}}}{(a-w)^{\frac{k_5}{k_6 a}+1}}\,dw\right\}$$

Simplifying as before and letting $\frac{k_5}{k_6 a} = n$; $\frac{k_3}{k_6} = p$

and $w = av$, the equation becomes

$$R = \left(\frac{1-v}{v}\right)^n \left(C + p\int \frac{v^n}{(1-v)^{n+1}}\,dv\right)$$

This equation cannot be solved as such for the least-square-set of constants because it is not linear in the constants. It can, however, be made linear in the following manner:

$$\text{Let } \left(\frac{1-v_i}{v_i}\right)^n \left(C + p\int \frac{v_i^n}{(1-v_i)^{n+1}}\right) = f_i(n,p)$$

Each v_i is a constant, one for each value of v given by the data.

A set of equations results.

Now, if an approximate set of values can be found for n and p, say $\bar{n}$ and $\bar{p}$, then set

$$n = \bar{n} + \alpha$$

$$p = \bar{p} + \beta,$$

where α and β may be considered as correction factors.

Now,

$$f_i(n,p) = f_i(\bar{n}+\alpha,\ \bar{p}+\beta) = f_i(\bar{n},\bar{p}) + \frac{\partial f_i(\bar{n},\bar{p})}{\partial \bar{n}}\,\alpha + \frac{\partial f_i(\bar{n}\bar{p})}{\partial \bar{p}}\,\beta$$

Now, the equations of conditions are

$$\frac{\partial f_i(\bar{n},\bar{p})}{\partial \bar{n}}\,\alpha + \frac{\partial f_i(\bar{n},\bar{p})}{\partial \bar{p}}\,\beta = R_D - f_i(\bar{n}\,\bar{p})$$

where R_D = the value of R from the data.

This equation is linear in α and β and can be solved by the method of least squares.

Now, since,

$$\frac{\partial f_i(np)}{\partial n} = \left(\frac{1-v}{v}\right)^n \mathrm{Log}_e\left(\frac{1-v}{v}\right)\left\{C + p\int \frac{v^n}{(1-v)^{n+1}}\,dv\right\}$$

$$+ \left(\frac{1-v}{v}\right)^n \left\{p\int \frac{v^n}{(1-v)^{n+1}}\,\mathrm{Log}_e\,\frac{v}{1-v}\,dv\right\}.$$

Then, when $\bar{n} = 1$, $\bar{p} = 28.375$ and $C = -\bar{p}$, we have

$$\frac{\partial f_i(\bar{n}\,\bar{p})}{\partial \bar{n}} = 28.375\left(\frac{1-v}{v}\right)\mathrm{Log}_e\left(\frac{1-v}{v}\right)\left\{\frac{v}{1-v} + \mathrm{Log}_e\,(1-v)\right\}$$

$$+ 28.375\left(\frac{1-v}{v}\right)\left\{\int \frac{v}{(1-v)^2}\,\mathrm{Log}_e\,\frac{v}{1-v}\,dv\right\}$$

The integral $\int \frac{v}{(1-v)^2} \text{Log}_e \frac{v}{1-v}\, dv$ can be evaluated by Simpson's rule and is given in table II. Also in table II are given the values of the integral $\int \frac{v}{(1-v)} \text{Log}_e \frac{v}{1-v}\, dv$ which is necessary if the equation for Case IV or Case VI is to be treated in a similar manner to Case V.

TABLE II

W	V	$\int \frac{v}{1-v} \text{Log}_e \frac{v}{1-v}\, dv$	$\int \frac{v}{(1-v)^2} \text{Log}_e \frac{v}{1-v}\, dv$
0	0	0	0
5	.08197	-.01031	-.01089
10	.16393	-.03267	-.03648
15	.24590	-.06112	-.07234
20	.32787	-.09091	-.11415
25	.40984	-.11631	-.15431
30	.49180	-.12905	-.17715
30.5	.50000	-.12918	-.17741
35	.57377	-.11584	-.14768
40	.65574	-.05286	+.01962
45	.73770	+.10804	+.56404
50	.81967	+.48366	2.32977
55	.90164	+1.46968	10.08239
60	.98361	+7.61661	77.17315

When n = 1

$$R = p\left(\frac{1-v}{v}\right)\left\{\frac{v}{1-v} + \text{Log}_e(1-v)\right\}$$

$$\therefore \frac{\partial f_i(n,p)}{\partial p} = \frac{\partial f_i(\overline{np})}{\partial \overline{p}} = \left(\frac{1-v}{v}\right)\frac{v}{1-v} + \text{Log}_e(1-v)$$

and, therefore, the equations of condition become

$$28.375\left(\frac{1-v}{v}\right)\text{Log}_e\frac{i-v}{v}\left\{\frac{v}{1-v}+\text{Log}_e(1-v)\right\}+28.375\left(\frac{1-v}{v}\right)$$

$$\left\{\int\frac{v}{(1-v)2}\,\text{Log}_e\,\frac{v}{1-v}\,dv\right\}\alpha+\left\{\left(\frac{1-v}{v}\right)\frac{v}{1-v}+\text{Log}_e(1-v)\right\}\beta$$

$$=R_D-f_i(\bar{n}\ \bar{p})$$

Solving for α and β, they are found to be

$$\alpha=-0.23157$$

$$\beta=-8.6106$$

therefore $n=0.7684$

$p=19.764$

and $$R=19.764\left(\frac{1-v}{v}\right)^{0.7684}\left(1-\int\frac{v^{0.7684}}{(1-v)1.7684}\,dv\right)$$

or $$R=f_i(\bar{n},\bar{p})+\frac{\partial f_i(\bar{n}\ \bar{p})}{\partial\bar{n}}\alpha+\frac{\partial f_i(\bar{n},\bar{p})}{\partial\bar{p}}\beta$$

The equation is plotted in figure 6. The sum of the squares of the differences is 106.51.

The equationfor Case VI is

$$R=\left(\frac{a-w}{w}\right)^{\frac{k_5}{k_6a}}\left\{C+\frac{k_1+k_3}{k_6}\int\frac{w^{\frac{k_5}{k_6a}}}{(a-w)^{\frac{k_5}{k_6a}}+1}\,dw+k_2\int\frac{w^{\frac{k_5}{k_6a}}}{(a-w)^{\frac{k_6}{k_5a}}}\,dw\right\}$$

which for purposes of least-square-fitting is identical with the equation for Case IV. The constants and form of the final equation is therefore the same and is

$$R=\frac{61-w}{w}\left\{31.0982\,\frac{w}{61-w}+\text{Log}_e\,\frac{61-w}{61}+26.261\,\frac{w}{61}+\text{Log}_e\,\frac{61-w}{61}\right\}$$

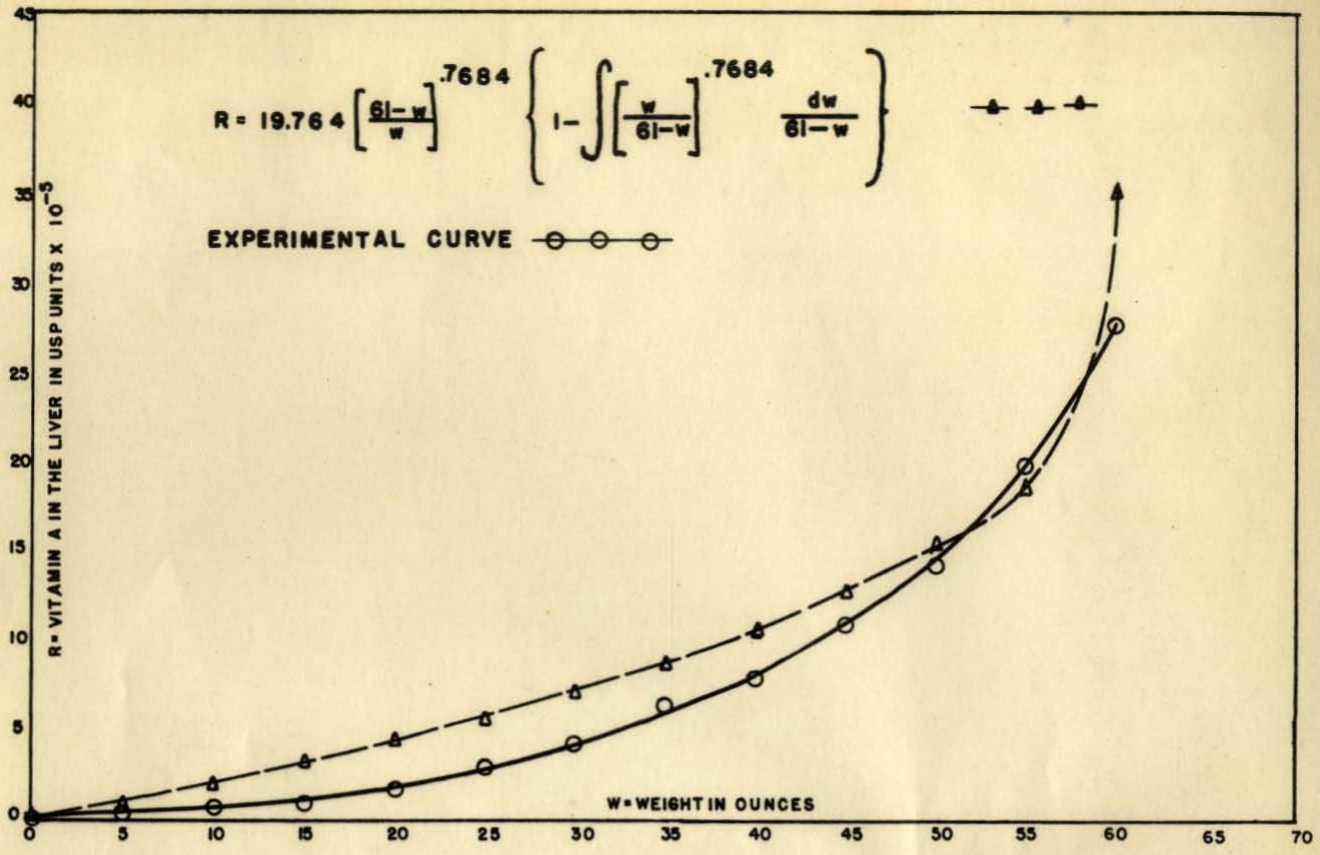

FIGURE 6

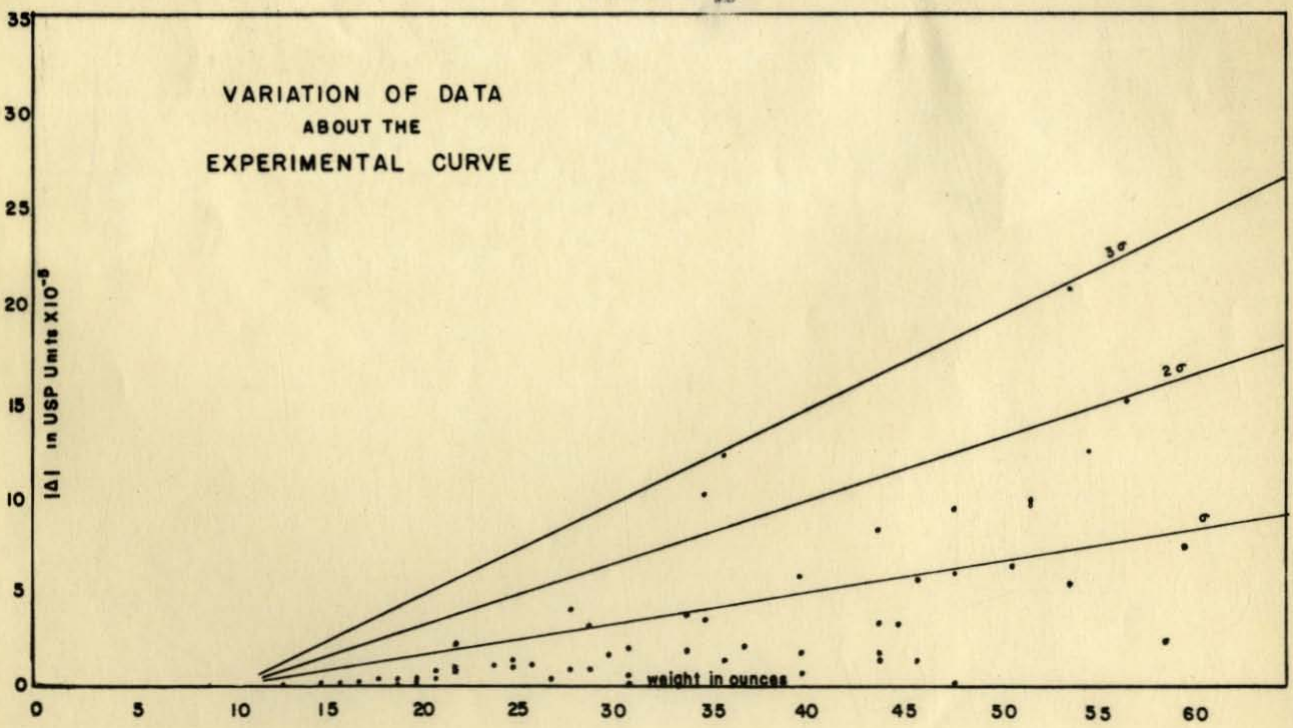

FIGURE 7

DISCUSSION

Of all the equations presented, the one derived for Case IV and Case VI gives by far the best fit. Case IV, the mechanism with storage by ingestion alone and the utilization in proportion to the reserve present in the liver, is the case which seems to have the most support in the literature to date for all animals investigated. It would seem then that fish in general and the brill in particular are no exception to this rule. At this point, however, it would be hazardous to state that either Case IV or Case VI was the mechanism in preference to the other cases. As it stands, there are not enough data to prove this conclusively.

In order to ascertain the number of fish necessary to determine the true mean curve to within 10,000 USP Units throughout the entire length of the curve with a probability of at least 95%, it is necessary to know approximately how the deviation from the mean varies with the age of the fish. Figure 7 shows the absolute value of the deviations plotted against increasing weight. The line marked 3σ was arbitrarily drawn to just include the greatest deviations from the mean. That this assumption is approximately correct is shown by Table III.

TABLE III

Deviation from mean	Normal Distribution		Graph	
	% of points	No. of points	% of points	No. of points
mean - 1σ	68.3	40	77.6	45
1σ - 2σ	27.2	16	17.2	10
2σ - 3σ	4.3	2	5.2	3

Table III shows that the arbitrary assumption made forces a larger proportion of the data closer to the mean than would be expected. For purposes of estimating the number of fish necessary for a further experiment this fact will result in the number of fish being too high, in other words the estimate will be a conservative one.

Now, setting 1 unit on the graph (10,000 USP Units) to be twice the standard deviation of the sample mean at any particular weight, we have

$$1 = \frac{2\sigma}{\sqrt{n}}$$

where σ is the estimated standard deviation of the population obtained from the graph and n is the sample size to be taken at the given weight.

$$n = (2\sigma)^2$$

TABLE IV

w	2σ	$(2\sigma)^2 = n$
60	16.0	256
55	14.5	210
50	12.7	162
45	11.1	124
40	9.5	91
35	8.0	64
30	6.3	40
25	4.6	21
20	3.0	9
15	1.5	2

Table IV shows the number of fish necessary to establish the mean within 10,000 USP Units with a probability of 95% for the corresponding weight. The number of fish "n" is higher than actually needed because each value obtained at any particular weight exerts an influence on the values obtained at the other weights

and, thereby, should decrease the sample size necessary. The problem of the exact size of sample needed is beyond the scope of this present paper. The conditions outlined above may be considered sufficient conditions but they are stronger than necessary.

Statistical treatment alone is not the only method of further attack on this problem. Most of the constants in the original postulates are capable of independent determination. Ideally, the problem could be solved by determining all of these constants from the postulates and comparing them with constants obtained from a statistical experiment with enough fish as outlined above.

CONCLUSIONS AND SUMMARY

(1) Six different hypotheses for the mechanism of vitamin A metabolism have been developed from theoretical postulates.

(2) These hypotheses have been compared with experimental results and the case which is supported most strongly by independent literature i.e. vitamin A storage by ingestion and utilization proportional to reserve present, is the case which gives the best fit of the data at hand.

(3) Since there are not sufficient data to make the argument statistically sound sufficient conditions for a further experiment which will do this are outlined.

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