4. In contrast to simple unbranched N-acyl derivatives of glycine, N-β-carboxypropionyl- and N-succinyl-glycine are hydrolysed *in vivo* to a negligible extent only. Corresponding derivatives of aminolaevulic acid also are not hydrolysed, nor is, in this case, the N-acetyl amino acid. With the latter compound rapid excretion may account for the failure of hydrolytic systems to yield the free amino acid.

5. Both the diester and the (α)mono-ethyl ester of amino-oxoadipic acid have been shown to give rise to porphobilinogen or porphyrins in biological systems. These included rats, liver homogenates, and suspensions or cell-free extracts of *Rhodopseudomonas spheroides*. This is not, however, sufficient evidence to establish that amino-oxoadipic acid is the biological precursor of aminolaevulic acid.

The authors wish to thank Dr June Lascelles, Microbiology Unit, Department of Biochemistry, Oxford, and Dr S. F. MacDonald, National Research Council, Ottawa, for making available information then unpublished. They also wish to acknowledge the technical assistance of Mr F. Grover, Mrs B. Higginson of the National Institute for Medical Research, and of Miss F. Wright of St Mary's Hospital Medical School.

REFERENCES

Abbott, L. D. (1942). *J. biol. Chem.* **145**, 241.
Arnstein, H. R. V. & Neuberger, A. (1951). *Biochem. J.* **50**, 154.
Bardham, J. C. (1936). *J. chem. Soc.* p. 1848.
Berlin, N. I., Neuberger, A. & Scott, J. J. (1956). *Biochem. J.* **64**, 80.
Christman, A. A. & Lewis, H. B. (1921). *J. biol. Chem.* **47**, 495.
Cookson, G. H. & Rimington, C. (1954). *Biochem. J.* **57**, 476.
Dresel, E. I. B. & Falk, J. E. (1953). *Nature, Lond.*, **172**, 1185.
Eisner, U., Elvidge, J. A. & Linstead, R. P. (1950). *J. chem. Soc.* p. 2223.
Gibson, K. D., Neuberger, A. & Scott, J. J. (1955). *Biochem. J.* **61**, 619.
Granick, S. & Vanden Schriek, H. G. (1955). *Proc. Soc. exp. Biol., N.Y.*, **88**, 270.
Harington, C. R. & Randall, S. S. (1931). *Biochem. J.* **25**, 197.
Jackson, R. W. & Chandler, J. P. (1939). *Annu. Rev. Biochem.* **8**, 249.
Lascelles, J. (1956). *Biochem. J.* **62**, 78.
MacDonald, S. F. & Stedman, R. J. (1955). *Canad. J. Chem.* **33**, 458.
Morgan, W. T. J. & Elson, L. A. (1934). *Biochem. J.* **28**, 988.
Neuberger, A. (1936). *Biochem. J.* **30**, 2085.
Neuberger, A. & Sanger, F. (1943). *Biochem. J.* **37**, 575.
Neuberger, A. & Scott, J. J. (1953). *Nature, Lond.*, **172**, 1093.
Neuberger, A. & Scott, J. J. (1954). *J. chem. Soc.* p. 1820.
Riegel, B. & Lilienfeld, W. M. (1945). *J. Amer. chem. Soc.* **67**, 1273.
Scheiber, J. & Reckleben, H. (1913). *Ber. dtsch. chem. Ges.* **46**, 2412.
Shemin, D. & Russell, C. S. (1953). *J. Amer. chem. Soc.* **75**, 4873.
Shemin, D., Russell, C. S. & Abramsky, T. (1955). *J. biol. Chem.* **215**, 613.
Shuster, L. (1956). *Biochem. J.* **64**, 101.

The Concentration of Glucose in Rat Tissues

By K. F. GEY
Department of Biochemistry, University of Oxford

(*Received* 17 *February* 1956)

Paper chromatography has been used in the present investigation for the determination of the true glucose content of eight tissues of the rat. This method is more effective than older methods in separating glucose from other reducing substances in tissue extracts, but it does not appear to have been used before for systematic investigations of the glucose content of animal tissues, though Wallenfels, Bernt & Limberg (1953) used it for measuring glucose in blood. Previous authors have determined the yeast-fermentable reducing substances in some tissues, and the values obtained by chromatography in the present work are of the same order.

EXPERIMENTAL

Preparation of tissues. Male albino rats, weighing 105–160 g., were used, after having been kept without food for 20–28 hr. but supplied with water *ad lib.* The animals were killed instantaneously either by immersion in liquid oxygen or by decapitation. In the latter method tissues were put in liquid oxygen after a specified time. The time interval between death and freezing is given in the first column of Tables 2–9. The tissues were broken up in the liquid oxygen with a pair of pliers and quickly weighed on a torsion balance while still frozen. The weighed tissue (up to 450 mg.) was ground in an ice-cold mortar with acid-washed sand in 1 ml. of 0·3 N-$Ba(OH)_2$ (Somogyi, 1945). The following additions were then made: 1 ml. of 0·3 N-$Ba(OH)_2$, water (2 ml. minus an allowance for tissue water, taken to be 80 % of the

tissue weight) and 2 ml. of $ZnSO_4$ (5% $ZnSO_4,7H_2O$, w/v). After the material had stood for 20 min. at room temperature it was filtered through a sintered-glass funnel (porosity grade 4). Blood was obtained from decapitated animals. It was heparinized and centrifuged. The plasma was stored for up to 2 hr. at 1–3° while the tissues were dealt with. For deproteinization 0·1 ml. of plasma was diluted to 2 ml. with water and 2 ml. of 0·3 N-$Ba(OH)_2$ and 2 ml. of $ZnSO_4$ were added.

Determination of total free reducing substances. The method of Nelson (1944) was applied to 1 ml. of filtrate of the material treated in this way. This procedure gave lower figures than were given by the method of Miller & Van Slyke (1936) or by deproteinization according to Folin & Wu (1919, 1922), followed by Nelson's method. It seems therefore to be more specific, and has the further advantage of giving good chromatographic separations. The deproteinization according to Cori, Closs & Cori (1933) with acidified $HgSO_4$ also proved suitable for subsequent chromatography, and gave about the same values, but it is more laborious.

A portion of the filtrate (1 ml.) was mixed with 1 ml. of copper mixture and 1 ml. of the arsenomolybdate reagent, as described by Nelson (1944). The spectrophotometer readings were made in a volume of 10 ml., usually at 660 mμ., but at 520 mμ. for higher concentrations. All readings were compared with standards treated identically.

Determination of glucose. A portion of the filtrate (2 ml.) was evaporated either at 40° by vacuum distillation or in a vacuum desiccator over $CaCl_2$ at 2 mm. Hg. The remaining filtrate (approx. 2 ml.) was also evaporated. The two residues were dissolved in 0·1 ml. of 0·001 N-HCl and quantitatively transferred (with one washing of 0·1 ml. of 0·001 N-HCl and one of 0·1 ml. of water) to 2 cm. strips of Whatman no. 1 paper. Up to 15 parallel 2 cm. strips were obtained from an 18¼ in. × 22½ in. Whatman paper sheet by cutting out segments of 1 cm. × 45 cm. so that the remaining strips were held together by a crossing strip at the top and bottom of the sheet. A stream of cool air was used to dry the solutions on the paper. The chromatographic solvent was *n*-propanol–ethyl acetate–water (7:1:2, by vol.) (Baar, 1954). The paper was developed by descending chromatography for 26–30 hr. and dried by warm air at 40–50°. The second strip was sprayed with benzidine–trichloroacetic acid (Bacon & Edelman, 1951) to locate the glucose. An area of about 2 cm. × 4 cm. corresponding to the glucose spot on the sprayed strip was cut out and extracted in 3 ml. of water at 60–70° for about 1 hr. The paper was partly disintegrated by vigorous shaking for a few seconds in the glass-stoppered tube. The cellulose fibres were then removed by filtration through sintered glass (porosity grade 4). Copper mixture (1 ml.) was added to 2 ml. of this filtrate and glucose was estimated colorimetrically as previously described. Glucose standards were run on parallel strips. Duplicate chromatographic estimations agreed within 4% if 20–200 μg. of glucose was present and within 7% if less than 20 μg. was present. The agreement between duplicates was within 10% when two pieces of tissue were analysed. Additions of 50–110 μg. of glucose to liver, kidney and brain were recovered within this limit of 10%. Results are expressed as mg. of glucose/100 g. of frozen tissue.

RESULTS

On average, 87% of the total reducing value for rat-blood plasma was found to be due to glucose (Table 1). A similar value was observed by Wallenfels *et al.* (1953) chromatographically for glucose in human-blood serum, and by earlier authors (Hiller, Linder & Van Slyke, 1925; Folin & Svedberg, 1926; Somogyi, 1927; Benedict, 1928; Molitor & Pollak, 1931) for yeast-fermentable substances in plasma or serum.

The values obtained for glucose and total reducing substances of various organs immediately after death, and a few minutes later, are given in Tables 2–9. As the results for the different time intervals after death were necessarily derived from different animals the variations observed represent not only the effect of differences in time but also of individual variability. The concentrations of glucose as well as

Table 1. *Total reducing substances and glucose in the blood plasma of rats*

Rat no.	Total reducing substances as glucose (mg./100 ml.)	Glucose (mg./100 ml.)	Glucose (% of total reducing substances)
28	61	59	97
10	82	67	82
8	76	71	93
12	111	85	77
30	124	110	89

Table 2. *Total reducing substances and glucose in the cerebral hemispheres of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	19	—	—	—
		20	8	40	—
2	0	22	8	36	—
		20	8	40	—
3	0·75	22	—	—	90
4	0·75	18	—	—	—
5	1·5	12	7	58	60
6	1·5	14	6	43	60
7	2	11	1	9	—

Table 3. *Total reducing substances and glucose in the testis of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	33	8	24	—
2	0	34	9	27	—
8	3	22	3	14	76
9	3	20	2	10	78
10	3·5	34	2	6	82
11	3·5	—	3	—	80

Table 4. *Total reducing substances and glucose in leg musle of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	54	22	41	—
2	0	45	22	49	—
8	1·5	59	34	57	76
9	1·5	52	24	54	78
10	1·5	55	34	62	82
11	1·5	39	17	44	80
12	2·5	44	31	70	85
13	2·5	55	—	—	104

of the total reducing substances were found to be very low in the cerebral hemispheres (Table 2). Glucose averaged 8 mg./100 g. and total reducing substances 20 mg./100 g. in the animals immersed in liquid oxygen. There are no data in the literature on the concentration of glucose, as opposed to total reducing substances, in the brain. The total reducing substances reported in earlier publications [all values from animals under Amytal (5-ethyl-5-*iso*pentylbarbituric acid) anaesthesia] were all higher: 33–71 mg./100 g. was found in the brains of starved dogs (Kerr & Ghantus, 1936), 35–37 mg./100 g. in the brains of starved rabbits (Kerr & Ghantus, 1936; Kerr, Hampel & Ghantus, 1937; Gurdjahn, Webster & Stone, 1949), and 74 mg./100 g. in the brains of unstarved cats (Klein, Hurwitz & Olsen, 1946; Klein & Olsen, 1947). The lower values for total reducing substances found in the rat brain may be due to species differences, or to differences in procedure.

The following considerations are relevant to the question whether the glucose values found in the animals killed by immersion in liquid air are identical with those occurring in the living animal. Richter & Dawson (1948) measured the time required to freeze the whole brain of a 40 g. rat placed in liquid air. They found that the temperature remained nearly normal for 9–20 sec. and then dropped quickly. As rats weighing 100 g. were used in the present experiments a slower rate of cooling is expected. If an assumption is made on the cooling rate of the tissue the amount of glucose consumed by the tissue can be calculated from known Q_{O_2} values, conditions being aerobic. It is assumed that the tissue remained at body temperature for 30 sec. The highest value reported, Q_{O_2}, -26 (Krebs, 1950), corresponds to a glucose consumption of 6 mg./100 g./0·5 min. There is no reason for assuming that the glucose supply from the blood ceases immediately on cooling, and 6 mg./100 g./0·5 min. may therefore be regarded as the maximum disappearance rate. The actual rate is probably smaller. If the maximum figure is added to the observed value of 8 mg./100 g., a glucose concentration of 14 mg./100 g. is obtained. The results in tissue frozen up to 2 min. after killing indicate that the fall of tissue glucose is certainly not greater than 6 mg./100 g./0·5 min. At least as much of the reducing substances other than glucose as of glucose was found in brain.

Testis also gave low values for glucose and total reducing substances, namely 8 mg./100 g. for glucose, and 25 mg./100 g. for reducing substances other than glucose (Table 3). LePage (1946*a*, *b*) found that 40 sec. was required to freeze completely the abdominal organs of a 300 g. rat placed in liquid air. As the rate of glucose consumption of testis is lower than that of brain [Q_{O_2}, -12 (Krebs & Johnson, 1948)], the correction for post-mortem changes may be taken to be at most 3 mg./100 g. As in brain, the glucose value fell to a very low level within 2 min. The changes of the reducing substances other than glucose were smaller and less uniform than those for glucose.

Tables 4 and 5 show the amount of glucose in 100 g. of muscle and diaphragm. In skeletal muscle

22 mg./100 g. was found in the quickly frozen animal, and somewhat more in four out of five muscles frozen 1·5–3 min. after death. Diaphragm shows a similar post-mortem increase of free glucose. The glucose concentrations found are in general agreement with the concentrations of yeast-fermentable substances reported by Power & Clawson, 1928; Bischoff & Long, 1932; Trimble & Cary, 1931, and somewhat higher than those found with Cori's method (Cori *et al.* 1933). The value for total reducing substances of diaphragm agrees with data of Krahl & Cori (1947). Lower values found by Bornstein & Park (1953) are probably due to differences in technique.

Spleen showed glucose concentrations similar to those of muscle (Table 6): 22–23 mg./100 g. was found in the animal quickly frozen in liquid oxygen, decreasing to 6 mg./100 g. within 3·5 min. after death. The concentration of non-reducing substances was very low.

The average glucose value in the kidney (mainly cortex) was 51 mg./100 g. in the first minute (Table 7), and the value for reducing substances other than glucose was low. Within 3·5 min. after death the glucose level dropped to 15 mg./100 g., but the total reducing substances did not change. This suggests that glucose is rapidly converted into another reducing substance. The rapid changes after

Table 5. *Total reducing substances and glucose in the diaphragm of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)
14	0·5	58	25	43
15	1	45	24	53
16	1	44	—	—
17	1·5	49	32	65

Table 6. *Total reducing substances and glucose in the spleen of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	23	22	96	—
2	0	24	23	96	—
10	1·5	15	13	87	82
18	2	34	25	73	92
19	2	17	15	88	90
12	3	10	8	80	85
13	3	17	14	82	104
8	3·5	9	6	67	76

Table 7. *Total reducing substances and glucose in the kidneys of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	55	51	93	—
2	0	57	55	96	—
18	1	51	43	84	92
19	1	54	54	100	90
12	1·5	51	43	84	85
13	1·5	44	38	86	104
20	1·5	52	39	75	—
21	1·5	54	40	74	—
8	2·5	50	37	74	76
9	2·5	43	36	84	78
22	2·5	40	29	72	—
23	2·5	42	29	69	—
10	2·5	39	22	57	82
11	3·5	45	15	33	80

Table 8. *Total reducing substances and glucose in the lungs of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	63	63	100	—
3	3	36	—	—	90
10	3	32	25	78	82
11	3	32	25	78	80

Table 9. *Total reducing substances and glucose in the livers of starved rats*

Rat no.	Time of freezing after death (min.)	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Glucose (% of total reducing substances)	Plasma glucose (mg./100 ml.)
1	0	93	88	95	—
2	0	90	85	95	—
24	0·5	99	85	86	—
25	0·75	99	93	94	—
8	0·75	84	82	98	76
9	0·75	85	75	88	78
10	0·75	80	73	91	82
11	0·75	72	71	99	80
26	2	198	132	67	—
27	2	182	160	88	—
12	2	270	189	70	85
13	2	252	256	102	104
3	2	209	173	83	90

Table 10. *Total reducing substances and glucose in rat tissues within one minute after death*

Average values calculated from Tables 2–9.

Tissue	Total reducing substances as glucose (mg./100 g.)	Glucose (mg./100 g.)	Non-glucose reducing substances as glucose (mg./100 g.)
Brain	20	8	12
Testis	34	9	25
Leg muscle	50	22	28
Diaphragm	49	25	24
Spleen	24	23	1
Kidney	54	51	3
Lung	63	63	0
Liver	88	82	6

death may be connected with the high respiration of this tissue. If glucose were the substrate of respiration 18 mg. would be used/100 g. of tissues in 1 min. (Q_{O_2}, −38).

In lung 63 mg. of glucose/100 g. of tissue was found in an animal killed by freezing (Table 8). Three minutes after decapitation 25 mg./100 g. was present. The value for reducing substances other than glucose was low.

In liver (Table 9) values for glucose and total reducing substances did not change within the first minute after death. The average was 82 mg./100 g. for glucose, and 88 mg./100 g. for the total reducing substances. Two minutes after death the concentrations of both the glucose and reducing substances other than glucose were substantially higher (see Table 9). An increase in sugar after death has been known since Dalton (1871) and has been ascribed to the breakdown of glycogen (Panormoff, 1893; Molitor & Pollak, 1931). Molitor & Pollak (1931) determined the yeast-fermentable substances in liver and found 83 mg./100 g. in unstarved dogs and 97 mg./100 g. in rabbits, values which are of the same order as the glucose values found chromatographically in rat liver within the first minute after death.

The results obtained from different organs are summarized in Table 10, which presents the average concentrations found within the first minute after death for all organs investigated.

The glucose levels of the tissue increase in the following ascending sequence: brain and testis, leg muscle, diaphragm and spleen, kidney, lung, liver. The levels of reducing substances other than glucose were higher than the glucose levels in brain and testis, about equal to the glucose level in muscle, and very low in the other four tissues.

DISCUSSION

The glucose levels of the eight rat tissues show remarkable differences, varying between an average of 8 mg./100 g. in brain and an average of 82 mg./100 g. in liver.

Low glucose values seem to parallel relatively high rates of glycolysis and relatively low vascularization, although spleen is again an exception. Gibson *et al.* (1946) assessed the blood content of dog brain and muscle at about 2% of the tissue weight, compared with 20% in kidney, lung and liver.

The glucose concentration as measured is the resultant of the concentrations in the different tissue spaces. The low glucose values for brain and testis suggest that the glucose concentration of the 'extracellular' space in these tissues is lower than that of plasma. As the size of the 'glucose space' is not known no precise calculations can be made.

The glucose content increased after death in liver and muscle, tissues known to contain appreciable amounts of glycogen, but fell in spleen, kidney and lung. The level of reducing substances other than glucose remained steady after death in those tissues which showed a relatively high initial value (brain, testis, muscle) and increased where the initial value was low (spleen, kidney, lung and liver).

SUMMARY

1. The true glucose content of eight rat tissues has been measured. Glucose was separated by paper chromatography and determined by the method of Nelson. The total reducing value was also determined.

2. When rats were killed by immersion in liquid oxygen, all the tissues except liver contained less glucose than did plasma, the value for brain and testis being only about one-tenth, and for muscle and spleen about a quarter, of the value for plasma.

3. The effects of delays in freezing the tissue after death were measured.

The author wishes to thank Professor H. A. Krebs, F.R.S., for his encouragement, help and advice. This work was supported by a personal grant from F. Hoffmann–La Roche and Co., Basle.

REFERENCES

Baar, S. (1954). *Biochem. J.* **58**, 175.
Bacon, J. S. D. & Edelman, J. (1951). *Biochem. J.* **48**, 114.
Benedict, S. R. (1928). *J. biol. Chem.* **76**, 457.
Bischoff, F. & Long, M. S. (1932). *J. biol. Chem.* **95**, 743.
Bornstein, J. & Park, C. R. (1953). *J. biol. Chem.* **205**, 503.
Cori, G. T., Closs, J. O. & Cori, C. F. (1933). *J. biol. Chem.* **103**, 13.
Dalton, J. C. (1871). *A Treatise of Human Physiology*, 5th ed. Philadelphia. [Cited by Palmer, W. W. (1917). *J. biol. Chem.* **30**, 79.]
Folin, O. & Svedberg, A. (1926). *J. biol. Chem.* **70**, 405.
Folin, O. & Wu, H. (1919). *J. biol. Chem.* **38**, 81.
Folin, O. & Wu, H. (1922). *J. biol. Chem.* **51**, 419.
Gibson, J. G., Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J. & Evans, R. D. (1946). *J. clin. Invest.* **25**, 848.
Gurdjahn, E. S., Webster, J. E. & Stone, W. E. (1949). *Amer. J. Physiol.* **156**, 149.
Hiller, A., Linder, G. C. & Van Slyke, D. D. (1925). *J. biol. Chem.* **64**, 625.
Kerr, S. E. & Ghantus, M. (1936). *J. biol. Chem.* **116**, 9.
Kerr, S. E., Hampel, C. W. & Ghantus, M. (1937). *J. biol. Chem.* **119**, 405.
Klein, J. R., Hurwitz, R. & Olsen, N. S. (1946). *J. biol. Chem.* **164**, 509.
Klein, J. R. & Olsen, N. S. (1947). *J. biol. Chem.* **167**, 747.
Krahl, M. E. & Cori, C. F. (1947). *J. biol. Chem.* **170**, 607.
Krebs, H. A. (1950). *Biochim. biophys. Acta*, **4**, 249.
Krebs, H. A. & Johnson, W. A. (1948). *Tabul. biol., Hague*, **29**, 100.
LePage, G. A. (1946*a*). *Amer. J. Physiol.* **146**, 267.
LePage, G. A. (1946*b*). *Amer. J. Physiol.* **147**, 446.
Miller, I. & Van Slyke, D. D. (1936). *J. biol. Chem.* **114**, 583.
Molitor, H. & Pollak, L. (1931). *Arch. exp. Path. Pharmak.* **162**, 488.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Panormoff, A. (1893). *Hoppe-Seyl. Z.* **17**, 596.
Power, M. H. & Clawson, T. A. jun. (1928). *J. biol. Chem.* **78**, lvi.
Richter, D. & Dawson, R. M. C. (1948). *Amer. J. Physiol.* **154**, 73.
Somogyi, M. (1927). *J. biol. Chem.* **75**, 33.
Somogyi, M. (1945). *J. biol. Chem.* **160**, 69.
Trimble, H. C. & Cary, B. W. jun. (1931). *J. biol. Chem.* **90**, 655.
Wallenfels, K., Bernt, E. & Limberg, G. (1953). *Angew. Chem.* **65**, 581.

Vitamin A_2 in Indian Fresh-water Fish-Liver Oils

BY S. BALASUNDARAM, H. R. CAMA, P. R. SUNDARESAN AND T. N. R. VARMA
Department of Biochemistry, Indian Institute of Science, Bangalore

(*Received* 28 *December* 1955)

The possible existence of a new chromogen showing an absorption band at 693 mμ. in the colour test with antimony trichloride was first postulated by Heilbron, Gillam & Morton (1931). A little later, Edisbury, Morton & Simpkins (1937) designated this new chromogen vitamin A_2, on the strength of the very close similarity in the distribution of vitamin A_1 (620 mμ. band, colour test) in the liver oils of marine fishes and of the new chromogen in most fresh-water fishes. Simultaneously, Lederer & Rosanova (1937), in an examination of liver oils from fishes of Russian rivers, observed the predominance of the 693 mμ. band over the 620 mμ. band of vitamin A_1. Its importance in the visual