THE CHEMISTRY OF GLUCONEOGENESIS.

II. THE FORMATION OF GLUCOSE FROM VALERIANIC AND HEPTYLIC ACIDS.

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In a previous communication[1] under this title, evidence was presented to the effect that in phlorhizinized animals, the administration of 10 grams of propionic acid, as ammonium or sodium salts, *per os* or subcutaneously, was followed by an increase in the glucose elimination which corresponded to all of the carbon of the propionic acid. The suggestion was then made that phlorhizinized animals have the power of quantitatively[2] synthesizing propionic acid into glucose. In this series of experiments the influence of the homologues of propionic acid are considered.

Methods

Female dogs were used in all the experiments. The urine was collected by catheter, and the bladder washed with distilled water at the end of every period. Merck's phlorhizin was used, and was injected in 2-gram doses three times per twenty-four hours, as recommended by Lusk.[3] The following methods were used in the analyses: nitrogen, Kjeldahl-Gunning; ammonia, Folin; total acetone, Huppert-Messinger; aceto-acetic acid, Embden; glucose, the Allihn gravimetric method, also by polarization after clarifying the urine with basic lead acetate; β-hydroxybutyric acid, determined by Magnus-Levy's method. The β-hydroxybutyric acid results are relative, not absolute, for it was found that a considerable amount of

[1] A. I. Ringer: The Chemistry of Gluconeogenesis. I. The Quantitative Conversion of Propionic Acid into Glucose, this *Journal*, xii, p. 511, 1912.

[2] By quantitative, in this case, we understand the utilization of the entire molecule for the synthesis of glucose.

[3] Graham Lusk: Phlorhizinglukosurie, *Ergeb. d. Physiol.*, xiii, p. 315, 1912.

phlorhizin was eliminated by the kidneys in the urine. This phlorhizin is extracted with the ether and has a specific rotation of $-42.3°$. The result of this investigation will be reported in the near future.

The effect of formic acid (HCOOH).

In the first table are tabulated the results of an experiment in which the animal received 11.5 grams of formic acid, subcutaneously, as sodium salt dissolved in 50 cc. of water. There was no increase in the glucose, nor any increase in the D : N ratio. In experiment V, period V, the animal received a similar amount of formic acid, with similarly negative results. These two experiments show that *the diabetic organism does not possess the power of utilizing formic acid in the synthesis of glucose.*

The effect of normal butyric acid (CH_3-CH_2-CH_2-COOH).

In experiment V, period III, the animal received subcutaneously, 10 grams of normal butyric acid (Merck) dissolved in 50 cc. of water and neutralized with sodium hydroxide. In experiment VI, period IV, the animal received 20 grams of the same butyric acid, administered in a similar manner. In neither experiment was there any increase in the glucose elimination. The aceto-acetic acid and β-hydroxybutyric acid, however, were increased quite markedly. During period V of experiment VI the animal was in deep coma, presenting all the typical symptoms of diabetic coma, and it ended in death.

The effect of normal valerianic acid (CH_3-CH_2-CH_2-CH_2-COOH).

In experiment VII, period II, 9.2 grams of normal valerianic acid (Kahlbaum), dissolved in 50 cc. of water and neutralized with sodium hydroxide, were administered subcutaneously. The glucose elimination, which was 19.23 grams in the first period, rose to 24.13, in spite of a lower nitrogen metabolism in that period. The D : N ratio, which normally has a tendency to sink with the progress of the glucosuria, rose from 3.38 in the first period to 5.05 and 3.97 in the second and third periods, respectively. It is evident therefore that "extra glucose" was eliminated during periods II and III which did not find its origin in the catabolized protein. As no analyses were made during period IV of that experiment, we are forced to assume that the D : N ratio in

periods II and III would have remained at 3.38 had no valerianic acid been given. In calculating the "extra glucose" an error is thus introduced which makes the results a little too low. The extra glucose can be calculated by the following formula: $EG = G - (N \times Q)$; $E\,G$ stands for extra glucose, G stands for the value of the glucose eliminated, N stands for the value of the nitrogen, Q stands for the value of the assumed D : N quotient.

G in periods II and III is equal to $24.13 + 18.54 = 42.67$ grams.
N in periods II and III is equal to $4.78 + 4.67 = 9.45$ grams.
Q in periods II and III is assumed to be 3.38.

$$E\,G = G - (N \times Q) = 42.67 - (9.45 \times 3.38) = 10.7.$$

9.2 grams of normal valerianic acid give rise to 10.7 grams of extra glucose.

In experiment IX, period II, the animal received 14.2 grams of normal valerianic acid administered as above. The glucose elimination rose from 18.28 grams in the fore period to 26.20 and 21.08 in the second and third periods respectively. The D : N ratio, which was 3.55 in the fore period, rose to 5.28 and 3.89 in the second and third periods.

Calculation of "extra glucose."

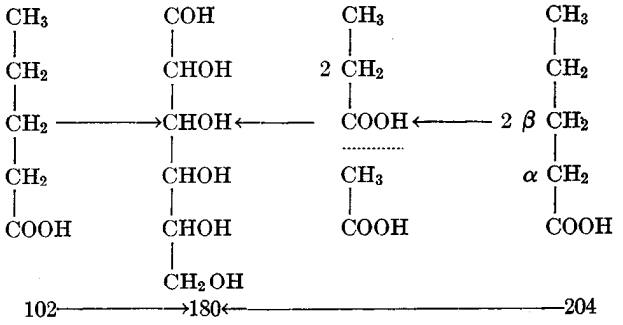
G in periods II and III is equal to $26.20 + 21.08 = 47.28$ grams.
N in periods II and III is equal to $4.96 + 5.41 = 10.37$ grams.
Q in periods II and III is assumed to be $\frac{3.55 + 3.64}{2} = 3.6$.

$$E\,G = G - (N \times Q) = 47.28 - (10.37 \times 3.6) = 9.95.$$

14.2 grams of normal valerianic acid give rise to 9.95 grams of extra glucose.

These two experiments agree in showing that normal valerianic acid can be utilized in the synthesis of glucose. Although the extent of the utilization differs widely in these two experiments, we are justified, from the very nature of experiments on phlorhizinized animals, in accepting the maximal figures as the ones which show the maximum extent of synthesis. In experiment VII we obtained 10.7 grams of glucose from 9.2 grams of normal valerianic acid. Calculated per 10.0 grams of valerianic acid, the yield of glucose is equal to 11.6 grams. In experiment IX, 14.2 grams of valerianic acid yielded 9.95 grams of glucose, which gives for 10.0 grams of this acid 7.0 grams of glucose. How can

we assume this glucose to have arisen from the valerianic acid molecule? A glance at the relationship of the valerianic acid molecule to the glucose molecule may throw light on this.



There are two possible ways in which valerianic acid may be converted into glucose. The first is by the utilization of all the carbons of valerianic acid in the upbuilding of the glucose molecule. In this case, 10.2 grams of the acid should give rise to 18.0 grams of glucose. This is not in accord with the findings in the experiments, and is besides highly improbable. The other possibility is that the products of decomposition of valerianic acid are synthesized into glucose. From the researches of Knoop, Embden, Dakin[4] and others, it is well established that the normal fatty acids in the animal body undergo oxidation and cleavage in the β-position. Valerianic acid would then give rise to propionic acid, which has already been shown by the writer[5] to be completely converted into glucose. Two molecules of valerianic acid would therefore be required for the formation of one molecule of glucose. Expressed in gram-molecular weight 10.2 grams of valerianic acid should give rise to 9 grams of glucose, which is in accordance with our findings. The conclusion is therefore justified that *valerianic acid gives rise to glucose in so far as it can give rise to propionic acid.*

The effect of normal caproic acid (CH_3-CH_2-CH_2-CH_2-CH_2-COOH).

In experiment VIII, period II, the animal received 9.2 grams of normal caproic acid (Kahlbaum) as sodium salt. In experiment

[4] H. D. Dakin: *Oxidations and Reductions in the Animal Body*, Longmans, Green and Company, 1912, contains the most recent review of the literature.

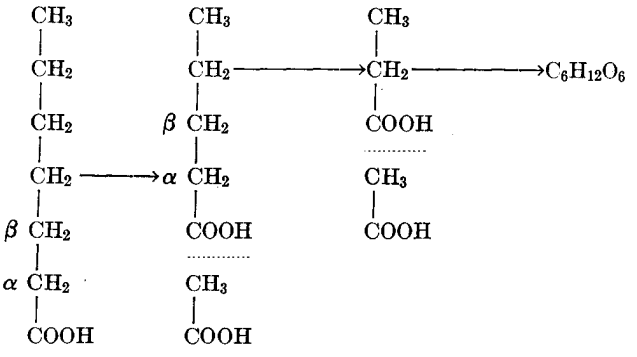
[5] *Loc. cit.*

IX, period V, the animal received 10.3 grams of the same acid. In neither case was there any increase in the glucose elimination. The acetone bodies, aceto-acetic acid and β-hydroxybutyric acid were increased considerably, especially in experiment IX. These two experiments corroborate the findings of earlier investigators[6] who found that caproic acid is a β-hydroxybutyric acid builder.

The effect of normal heptylic acid
(CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-COOH).

In experiment IX, period VII, 13 grams of normal heptylic acid were administered subcutaneously. The glucose elimination in that period was increased, but to a lesser extent than after valerianic acid feeding. Assuming that all of the extra glucose came out in that period, then $E\ G = G - (N \times Q) = 19.15 - (4.13 \times 3.75) = 3.67$ grams of extra glucose. In experiment X, period II, 13 grams of heptylic acid were administered as above. The glucose rose from 15.63 in the fore period to 20.99; the D : N ratio rose from 3.71 to 4.20.

$E\ G = G - (N \times Q) = 20.99 - (5.0 \times 3.78) = 2.09$ grams of extra glucose. The amount of glucose derived from heptylic acid is indeed small, but an examination of the table convinces one that there *is* a decided increase, and that it can come from no other source. The heptylic acid no doubt undergoes oxidation and cleavage in the β-position, being converted into valerianic acid and finally into propionic acid, and this fraction of the heptylic acid molecule goes over into glucose.



[6] H. D. Dakin: *loc. cit.*

The yield of glucose in these two experiments was not as great as might theoretically be expected. Further experiments will be performed soon, with the hope of establishing a more exact quantitative relationship.

It is very evident from these experiments that, while the fatty acids with the even number of carbons, as butyric and caproic acids, give rise to aceto-acetic acid and β-hydroxybutyric acid, those with an uneven number of carbons give rise to glucose. The amount of glucose that arises diminishes with the size of the fatty acid molecule, and there is every reason to believe that it is only the three final carbons which contribute to the glucose formation. If this is proven to be true in the case of the higher fatty acids with C_{15} and C_{17}, it may be of the utmost value in the treatment of diabetes mellitus.

It is now very well established that aceto-acetic acid and β-hydroxybutyric acid find their origin mainly in the catabolism of the fatty acids with an even number of carbon atoms. 256 grams of palmitic acid in severe cases of diabetes may give rise to as much as 104 grams of β-hydroxybutyric acid. Should we find that the fats containing C_{15} or C_{17} (which are in progress of preparation now) are burned in the body, we may thus have a very efficacious means of combating acidosis. From the above experiments we may rightly conclude that the fatty acids with an uneven number of carbons undergo oxidation in the β-position, and thus they *cannot* possibly give rise to acids with an even number of carbons.

Very interesting, also, is the fact that, in every case in which gluconeogenesis takes place, it is associated with a coincident antiketogenic process. It is illustrated very markedly in the case of the propionic acid feeding.[7]

Experiments on the different phases of this problem and their bearing on diabetes are being carried on, and I beg to be permitted to reserve this field of research for a reasonable length of time.

[7] A. I. Ringer: *loc. cit.*

SUMMARY.

Experiments were performed on phlorhizinized dogs and the glucose, nitrogen, ammonia, acetone, aceto-acetic acid and β-hydroxybutyric acid eliminations were studied.

I. The administration of formic acid is not followed by any increase in the glucose elimination.

II. Butyric and caproic acids produce an increase in the aceto-acetic and β-hydroxybutyric acid eliminations, but no increase in the glucose.

III. Valerianic and heptylic acids give rise to glucose, in all probability because of the formation of propionic acid as an intermediary body in their catabolism, after undergoing β-oxidation.

IV. Attention is called to the fact that the fatty acids with an uneven number of carbon atoms give rise to glucose.

The experimental data will be found in the following tables.

Dec. EXPERIMENT IV. *Twelve-hour periods.*

DATE 1912	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D:N	REMARKS
22	I	10.0	5.41	20.26	3.74	
22	II		**5.08**	**18.50**	**3.64**	11.5 grams formic acid as Na salt given subcutaneously in 50cc.
23	III		5.27	17.29	3.28	
23	IV		5.05	16.72	3.31	

Dec. — EXPERIMENT V. *Twelve-hour periods.*

DATE 1912	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D:N	NH_3N	ACETONE AND ACETO-ACETIC ACID	β-HYDROXY BUTYRIC ACID	REMARKS
14	I	13.3	5.62	25.68	4.57	0.21	0.109	0.686	
14	II		5.50	23.69	4.31	0.22	0.164	0.907	
15	III	13.0	**5.27**	**24.63**	**4.67**	**0.056**	**0.360**	**2.09**	10.0 grams normal butyric acid (Merck) as Na salt given subcutaneously in one injection.
15	IV		5.34	21.35	4.00		0.162	1.16	
16	V	12.8	**5.85**	**22.15**	**3.78**		**0.155**	**1.81**	11.5 grams formic acid as Na salt given subcutaneously.
16	VI		5.42	20.27	3.74		0.098	2.53	

Dec. — EXPERIMENT VI. *Twelve-hour periods.*

1	I	11.2	6.84	25.92	3.79	0.536	0.348		
1	II		6.78	22.51	3.17		0.372	2.47	
2	III		6.38	21.00	3.29	0.41	0.336	2.44	
2	IV	9.5	**6.18**	**20.82**	**3.37**	**0.18**	**0.599**	**3.30**	20.0 grams normal butyric acid as Na salt given subcutaneously in two doses.
3	V	Dog in deep coma. Exitus.							

Nov. — EXPERIMENT VII. *Twelve-hour periods.*

2	I	13.07	5.68	19.23	3.38	0.17	0.266	1.38	
3	II		**4.78**	**24.13**	**5.05**	**0.158**	**0.227**	**1.50**	9.2 grams normal valerianic acid as Na salt given subcutaneously.
3	III	12.2	**4.67**	**18.54**	**3.97**	**0.32**	**0.324**	**1.71**	

Oct. EXPERIMENT VIII. *Twelve-hour periods.*

DATE 1912	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D:N	NH_3N	ACETONE AND ACETO-ACETIC ACID	β-HYDROXY BUTYRIC ACID	REMARKS
7	I	17.00	7.14	28.04	3.92	0.308	0.147	0.692	
7	II		**7.12**	**27.30**	**3.84**	**0.33**	**0.275**	**1.24**	9.6 grams normal caproic acid as Na salt given subcutaneously.
8	III	16.46	6.65	25.93	3.89	0.35	0.575	2.13	

Nov. EXPERIMENT IX. *Twelve-hour periods.*

10	I	16.1	5.14	18.28	3.55	0.288	0.225	0.86	
10	II		**4.96**	**26.20**	**5.28**	**0.139**	**0.100**	**1.41**	14.2 grams normal valerianic acid as Na salt dissolved in 50 cc. of water given subcutaneously in one dose.
11	III		**5.41**	**21.08**	**3.89**	**0.162**	**0.247**	**1.80**	
11	IV	14.70	5.35	19.48	3.64	0.198	0.293	2.74	
12	V		**4.85**	**18.82**	**3.88**	**0.160**	**0.684**	**3.13**	10.3 grams normal caproic acid as Na salt given subcutaneously.
12	VI	13.80	4.89	17.86	3.65	0.181	0.76	4.32	
13	VII		**4.13**	**19.15**	**4.63**	**0.153**	**0.415**	**3.35**	13.0 grams normal heptylic acid as Na salt dissolved in 75 cc. of water.
13	VIII	13.24	3.72	14.38	3.86	0.188	0.564	Lost	
14	IX		4.20	14.84	3.53	0.242	0.948	3.83	
14	X		3.90	14.79	3.79	0.220			

Dec. EXPERIMENT X. *Twelve-hour periods.*

DATE 1912	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D:N	NH_2N	ACETONE AND ACETO-ACETIC ACID	β-HYDROXY BUTYRIC ACID	REMARKS
9	I	8.46	4.21	15.63	3.71	0.170		0.96	
9	II		**5.00**	**20.99**	**4.20**	**0.106**		**1.91**	13.0 grams normal heptylic acid as Na salt given subcutaneously in two doses.
10	III		4.26	16.44	3.86	0.202		3.13	
10	IV		4.43	16.94	3.82	0.200		3.58	
11	V		4.18	15.23	3.64			2.84	