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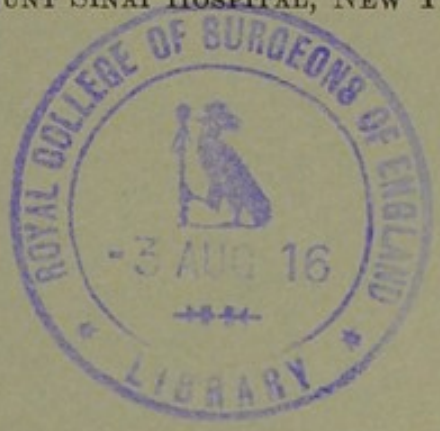

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STUDIES ON THE FORMATION OF GLYCOCOLL IN THE BODY. III.

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The evidence gained from recent studies on the formation of glycocoll, leads to the conclusion that after the administration of benzoic acid to an animal, a considerable part of the glycocoll eliminated as hippuric acid, is due to a synthesis of the amino-acid from simple substances.

The belief that leucine, as a representative of the higher amino-acids, contributes to the formation of glycocoll by cleavage, has been subjected to experimental proof, but with inconclusive results. It seems that the increased glycocoll output which follows the administration of benzoyl-leucine, is due not to a direct cleavage of the leucine radical, but in all probability to a resynthesis of the simpler substances into which the leucine is decomposed. At least no other explanation is available for the difference in the results obtained with pure leucine and benzoic acid on the one hand, and with benzoyl-leucine on the other.[1]

From the experiments previously reported by the authors,[2] it appears that the benzoyl radical of benzoyl-leucine possesses the power to couple more glycocoll within a given time than an equivalent amount of benzoic acid. Moreover, it is found that the administration of benzoic acid with leucine causes a smaller elimination of hippuric acid than benzoic acid alone is capable of producing. The diminution of the hippuric acid output consequent upon the administration of leucine is not accompanied by a decrease in the general protein metabolism as is that observed upon

[1] Epstein and Bookman: this *Journal*, xiii, p. 117, 1912, Magnus-Levy: *Münch. med. Wochenschr.*, lii, p. 2168, 1905.

[2] Epstein and Bookman: *loc. cit.*

feeding carbohydrates. The latter, as pointed out in an earlier paper,[3] lessens the total nitrogenous metabolism, without interfering with the efficiency of the benzoic acid to cause the production of glycocoll.

In the present investigation an attempt is made to ascertain three points:

1. The effect of feeding a simple amino-acid (alanine), alone and together with benzoic acid, upon the general protein metabolism and the formation of glycocoll.

2. The effect of feeding benzoyl-alanine upon the same phenomena.

3. The mechanism involved in the metabolism of a benzoylated nitrogen-free substance, and the formation of glycocoll.

As in our previous experiments, the tests were made on rabbits. Owing to the peculiar nature and the slight solubility of the benzoyl compounds (benzoyl-alanine and benzoyl-glucose) these substances were administered in powder form, *per os*. Certain errors may have crept in from this method of experimentation, but the uniformity of the results obtained justifies the belief that errors, if any, are negligible.

The first experiment comprises two series of nine three-day periods, in which the effect of benzoic acid and *i*-alanine singly and jointly was studied (condensed tables 1 and 2). The effect of feeding benzoyl-alanine was also tested.

As in our previous experiments, so too in the present tests it was found that the simple administration of benzoic acid causes a marked rise in the elimination of hippuric acid (tables 1 and 2, period 2). The administration of alanine alone causes a rise in the total nitrogen output (equivalent to the nitrogen of the alanine), but is without effect upon the hippuric acid output (cf. periods 3 and 4, tables 1 and 2). The simultaneous administration of alanine and benzoic acid causes a greater rise in the total urinary nitrogen, than that caused by alanine alone. The increment is equivalent to the sum total of the alanine-nitrogen and the extra nitrogen eliminated as hippuric acid (tables 1 and 2, period 6). Although the hippuric acid output in this last period (table 1, period 6) is greater than it is in the control benzoic acid period (2), *i.e.*, 0.141 as compared with 0.122 gram of nitrogen, the difference

[3] Epstein and Bookman: this *Journal*, x, p. 353, 1911.

CONDENSED TABLE 1.

Daily average for each period.

PERIOD	WEIGHT	FOOD (CARROTS)	BENZOIC ACID	ALANINE	ALANINE N	BENZOYL ALANINE	BENZOIC EQUIVALENT	URINE		
								Total N	Hippuric N	Benzoic equivalent
	grams	*grams*	*grams*	*gram*	*gram*	*grams*		*grams*	*gram*	*grams*
1	2060	300						1.166	0.010	0.083
2	2050	300	2.0					0.934	0.122	1.059
3	2160	300						0.770	0.016	0.136
4	2100	300		1.0	0.149			0.917	0.023	0.203
5	2140	300						0.719	0.011	0.093
6	2140	300	2.0	1.0	0.149			1.094	0.141	1.233
7	2000	300						0.545	0.022	0.191
8	1950	300				2.9	1.885	1.111	0.120	1.044
9	1550	300						1.081	0.025	0.217

Animal died accidentally.

CONDENSED TABLE 2.

Daily average for each period.

PERIOD	WEIGHT	FOOD (CARROTS)	BENZOIC ACID	ALANINE	ALANINE N	BENZOYL ALANINE	BENZOIC EQUIVALENT	URINE		
								Total N	Hippuric N	Benzoic equivalent
	grams	*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*	*gram*	*grams*
1	2000	300						0.585	0.017	0.151
2	2000	300	2.0					1.190	0.132	1.146
3	2020	300						0.991	0.016	0.146
4	2000	300		1.0	0.149			1.066	0.018	0.153
5	1960	300						0.583	0.009	0.077
6	1960	210	2.0	1.0	0.149			1.344	0.115	1.002
7	1940					2.9	1.885	1.539	0.051	0.450

is not sufficient to allow any other interpretation, as is shown by the results of the corresponding experiment in table 2.

After another interval of three days benzoyl-alanine was fed to the animal. This substance was given in amounts of 2.9 grams daily,[4] which we found to contain an amount of nitrogen equivalent to that of the alanine of the control tests.

The rise in the total urinary nitrogen in this period (8) corresponds with that obtained in period 6, table 1, in which benzoic acid and alanine were given simultaneously. The amount of hippuric acid, however, is less than in the control period (6, table 1) but corresponds to the period in which benzoic acid alone was given. It appears from this experiment that the benzoyl alanine does not produce more glycocoll than a corresponding amount of benzoic acid. It may therefore be said that alanine, free or in combination with the benzoyl radical, does not increase the output of glycocoll. The results show also that the benzoyl radical in benzoyl-alanine (unlike that of the benzoyl-leucine) fails to produce a greater output of hippuric acid than benzoic acid.

In order to test the efficiency of the benzoyl radical in a benzoyl compound to cause the production of glycocoll, the following experiment was performed. A rabbit, weighing 2400 grams, was given 2.0 grams of benzoic acid as sodium benzoate, daily, for three days and the hippuric acid output determined. After a normal interval of three days the animal was given 1.1 grams of glucose daily for four days, and the quantity of hippuric acid eliminated was also ascertained. Following this the rabbit received 2.0 grams of benzoic acid and 1.1 grams of glucose daily, and the hippuric acid elimination determined. After another normal interval of three days, this rabbit received 3.1 grams of benzoyl-glucose daily for a period of three days. This quantity of the substance is equivalent to 2.0 grams of benzoic acid and 1.1 grams of glucose.

The results of this experiment show that the benzoyl compound does not possess any greater power to produce and to couple glycocoll than benzoic acid alone (condensed table 3). In fact it appears that the elimination of hippuric acid in the benzoyl period is slightly less than in the benzoic acid period.

[4] It was found upon analysis that 2.9 grams of benzoyl-alanine represent 1.015 grams of alanine.

CONDENSED TABLE 3.

Daily average for each period.

PERIOD	WEIGHT	FOOD (CARROTS)	BENZOIC ACID	GLUCOSE	BENZOYL GLUCOSE	BENZOIC EQUIVALENT	URINE		
							Total N	Hippuric N	Benzoic equivalent
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*gram*	*gram*	*grams*
1	2400	300					0.843	0.020	0.136
2	2430	300	2.0				0.911	0.144	1.256
3	2270	300						Urine lost	
4	2200	300		1.1			0.574	0.033	0.290
5	2300	200	2.0	1.1			0.765	0.081	0.702
6	2260	285					0.542	0.011	0.097
7	2175	300		1.03	2.9	1.86	0.714	0.039	0.338
8	2190	300					0.639	0.013	0.120

In our experiments with benzoyl-leucine previously reported, we have observed that the increased glycocoll output in the benzoyl period was greater than could be ascribed to the leucine radical. We have attributed this result to a possibly greater efficiency of the benzoyl radical to couple glycocoll. It appears now from the experiment described above that the results obtained in the previous experiments were probably due to the presence of a greater amount of available glycocoll.

SUMMARY.

The data obtained in these experiments show that alanine, free or combined with a benzoyl radical, fails to yield glycocoll. This result is in accord with work of Magnus-Levy.[5] From the structural character of alanine it is not conceivable that it can be directly converted into glycocoll. Our results serve to show, however, that in its decomposition in the body, alanine does not yield cleavage products which can be synthesized into glycocoll. Furthermore, alanine has no direct or indirect effect on hippuric acid metabolism.

The increased output of hippuric acid, and hence glycocoll, observed after the administration of benzoyl-leucine is probably not a function of the benzoyl radical of the compound.

[5] Magnus-Levy: *loc. cit.*

TABLE 1.

DATE	WEIGHT	FEED CARROTS	BENZOIC ACID	ALANINE	ALANINE N	BENZOYL ALANINE	BENZOIC EQUIVALENT	ALANINE N EQUIVALENT	DAILY TOTAL N	TOTAL N FOR PERIOD	TOTAL HIPPURIC N FOR PERIOD	EXTRA HIPPURIC N	BENZOIC EQUIVALENT OF HIPPURIC ACID FORMED
	grams	*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*gram*	*grams*	*grams*	*gram*	*gram*	*grams*
Nov. 10–11	2040	300							1.169				
11–12	2070	300							1.113	3.500	0.029		0.250
12–13	2070	300							1.218				
14–15	2010	300	2.0						1.114				
15–16	2040	300	2.0						0.791	2.801	0.365	0.336	3.176
16–17	2100	300	2.0						0.896				
17–18	2180	300							0.616				
18–19	2170	300							0.840	2.310	0.048	0.019	0.410
19–20	2140	300							0.854				
20–21	2100	300		1.0	0.149				0.987				
21–22	2100	300		1.0	0.149				1.036	2.751	0.070	0.041	0.610
22–23	2100	300		1.0	0.149				0.728				
23–24	2140	300							0.896				
24–25	2100	300							0.490	2.156	0.032	0.003	0.280
25–26	2180	300							0.770				
26–27	2130	300	2.0	1.0	0.149				1.029				
27–28	2120	300	2.0	1.0	0.149				1.106	3.281	0.424	0.395	3.690
28–29	2070	300	2.0	1.0	0.149				1.146				
29–30	2020	300							0.560				
30– 1	2000	300							0.525	1.645	0.066	0.037	0.574
Dec. 1– 2	1970	300							0.560				
2– 3	2020	300				2.9	1.885	0.152	1.750				
3– 4	2000	300				2.9	1.885	0.152	0.952	3.323	0.360	0.331	3.133
4– 5	1740	300				2.9	1.885	0.152	0.630				
5– 6	1600	300							0.630	2.163	0.050		0.435
6– 7	1540	300							1.533				

TABLE 2.

DATE	WEIGHT	FEED CARROTS	BENZOIC ACID	ALANINE	ALANINE N	BENZOYL ALANINE	BENZOIC EQUIVALENT	ALANINE N EQUIVALENT	DAILY TOTAL N	TOTAL N FOR PERIOD	TOTAL HIPPURIC N FOR PERIOD	EXTRA HIPPURIC N	BENZOIC EQUIVALENT OF HIPPURIC ACID FORMED
	grams	*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*gram*	*grams*	*grams*	*gram*	*gram*	*grams*
Nov. 11–12	2000	300							0.609				
12–13	2020	300							0.490				
13–14	1950	300							0.553	2.765	0.088		
14–15	2040	300							0.420				
15–16	2000	300							0.693	1.659	0.052*		0.453
16–17	2020	300	2.0						1.120				
17–18		300	2.0						1.176	3.570	0.396	0.344	3.447
18–19	2080	300	2.0						1.274				
19–20	2070	300							0.952				
20–21	2000	300							0.938	2.975	0.050		0.440
21–22	2000	300							1.085				
22–23	2000	300		1.0	0.149				0.854				
23–24	1970	300		1.0	0.149				1.064	3.198	0.055		0.460
24–25		300		1.0	0.149				1.280				
25–26	1920	300							0.560				
26–27	2000	300							0.595	1.749	0.028		0.230
27–28	1920	300							0.594				
28–29	2000	300	2.0	1.0	0.149				1.394				
29–30	1920	300	2.0	1.0	0.149				lost	2.688	0.346	0.294	3.007
30– 1	1980	30	2.0	1.0	0.149				1.294				
Dec. 1– 2	2020	220							0.560				
2– 3	2020	300							0.574	1.674	0.081	0.039	0.700
3– 4	1920	300							0.560				
4– 5	1940					2.9	1.885	0.152	0.539		0.051		0.450

* For three days.

TABLE 3.

DATE	WEIGHT	FEED CARROTS	BENZOIC ACID	GLUCOSE	BENZOYL GLUCOSE	BENZOIC EQUIVALENT	DAILY TOTAL N	TOTAL N FOR PERIOD	TOTAL HIPPURIC N	BENZOIC EQUIVALENT OF HIPPURIC ACID FORMED
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*gram*	*grams*
Nov. 14–15	2410	300					1.183			
15–16	2410	300					0.896			
16–17	2430	300					0.931			
17–18	2390	300					0.644			
18–19	2400	300					0.777	2.529	0.057	
19–20	2400	300					0.630			0.510
20–21	2400	300	2.0				0.840			
21–22	2470	300	2.0				0.910		0.433	3.767
22–23	2450	300	2.0				0.882			
23–24	2340	300								
24–25	2240	300								
25–26	2220	300		1.1			0.549	2.369		
26–27	2210	300		1.1			0.630			
27–28	2140	300		1.1			0.497	1.722*	0.100*	0.870
28–29	2200	300		1.1			0.693			
29–30		300	2.0	1.1			0.553			
30– 1	2320	150	2.0	1.1			0.889	2.296	0.242	2.105
Dec. 1– 2		150	2.0	1.1			0.854			
2– 3	2270	260					0.463			
3– 4	2280	300					0.651	1.625	0.034	0.290
4– 5	2220	300					0.511			
5– 6	2220	300		1.1	3.1	2.0	0.553			
6– 7		300		1.1	3.1	2.0	0.791	2.142	0.117	1.017
7– 8	2130	300		0.9	2.5	1.613	0.798			
8– 9	2170	300					0.658			
9–10	2200	300					0.469	1.918	0.038	0.321
10–11	2200	300					0.791			