XCI. THE ABSORPTION OF MILK PRECURSORS BY THE MAMMARY GLAND.

III. THE RELATION OF AMINO-ACID ABSORPTION TO PROTEIN SYNTHESIS.

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THE work of Crowther and Raistrick [1916] indicates that the globulin of milk is identical with the globulin of blood. Lactalbumin and caseinogen appear, however, to be specific products of mammary secretion. Cary [1920], using the technique of Kaufmann and Magne [1906], demonstrated that the amino-acid content of blood obtained from the mammary vein of lactating cows was significantly lower than that obtained from the jugular vein. From this fact he concluded that the amino-acids of the blood are the precursors of the milk-proteins.

In view of the work of Delaunay [1913], Folin and Denis [1912], and Van Slyke and Meyer [1912] on the rôle of amino-acids in protein metabolism, such an assumption is *a priori* a reasonable one. Cary's conclusions are, however, open to criticism since he arbitrarily assumed that the amino-acid content of jugular blood could be taken as representative of the arterial blood flowing to the mammary gland. In the first paper of this series [Blackwood and Stirling, 1932, 2] it has been shown that the intervention of salivary secretion in the path of blood traversing the tissues of the head and neck necessitates a correction in the analytical values for jugular blood. In addition, no comparative values for the amino-acid contents of jugular and arterial bloods are to be found in the literature, so that Cary's assumption that these are identical appears unwarranted.

Apart from these criticisms, the method of blood analysis used by Cary was long and tedious, and involved so many separate processes (including incubation with urease, deproteinisation with boiling dilute acetic acid, treatment with kaolin, and subsequent concentration *in vacuo*) that considerable errors might be anticipated.

In view of these facts it appeared desirable to re-investigate the whole problem, using methods which were not open to the above criticisms. The methods adopted in the present investigation differ in three essentials from

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those of Cary. Estimations have been made of iron content of the blood samples, so that correction might be allowed for the water absorbed from the blood stream by the salivary glands. Samples of arterial blood have been taken in addition to samples of mammary and jugular venous blood so that direct comparisons could be obtained. Lastly, the manometric method of Van Slyke [1929] has been used for the estimation of amino-acid-nitrogen directly in Folin-Wu blood-filtrates. This method has been found to give rapid and accurate results.

EXPERIMENTAL.

Blood samples were taken from lactating and non-lactating cows as previously described [Blackwood and Stirling, 1932, 2]. Duplicate amino-acid-nitrogen and urea determinations were carried out according to the manometric methods of Van Slyke [1927, 1929]. Iron was estimated iodimetrically [Blackwood and Stirling, 1932, 1]. Corpuscular volumes were estimated by centrifuging to constant volume in haematocrit tubes.

In Table I are given the results of a preliminary series of observations carried out to ascertain if the differences between jugular and mammary bloods in amino-acid-nitrogen content reported by Cary could be detected by these methods and established as normal findings.

Table I.

No. of cow	Lactating or non-lactating	Date of analysis	Urea-nitrogen; mg. N per 100 cc. of blood		Amino-acid-nitrogen mg. N per 100 cc. of blood		Difference. Jugular- mam- mary %
			Jugular	Mammary	Jugular	Mammary	
1	Lactating	27. x. 30	12.12	12.93	7.036	6.739	4.2
2	"	4. xi. 30	10.02	10.04	5.884	4.825	18.0
3	"	10. xi. 30	7.192	6.969	6.420	5.768	10.1
4	"	13. xi. 30	9.619	9.529	7.375	6.324	14.2
5	"	17. xi. 30	9.72	9.04	7.257	6.276	13.5
6	"	20. xi. 30	10.76	10.58	6.087	5.059	16.9
6	"	26. x. 31	6.09	6.44	8.314	7.863	5.4
7	"	24. xi. 30	11.49	11.31	7.593	6.145	19.0
7	"	1. iii. 32	10.92	11.34	8.129	6.900	15.2
8	"	27. xi. 30	13.57	13.475	7.551	6.792	10.0
9	"	3. xii. 30	11.472	11.290	6.985	6.945	0.6
10	"	8. xii. 30	9.53	9.709	7.290	6.210	14.8
10	"	2. ii. 31	10.85	10.10	8.357	7.489	13.9
11	"	15. xii. 30	11.51	11.69	7.695	6.857	10.9
12	"	19. iii. 31	11.00	11.00	5.479	4.785	12.6
12	"	14. ix. 31	10.08	9.24	7.554	6.754	15.9
2	Non-lactating	19. i. 31	8.01	7.83	5.771	5.790	- 0.3
12	"	14. i. 31	9.95	9.77	5.922	5.934	- 0.2
12	"	22. i. 31	10.20	9.77	5.949	5.934	0.2
13	"	9. xi. 31	10.85	11.06	6.925	7.187	- 3.6
14	"	23. xi. 31	5.46	6.34	6.556	6.643	- 1.3
15	"	26. iii. 31	8.62	8.56	5.676	5.718	- 0.8

Table II gives the results of a second series in which complete analytical results (jugular, arterial and mammary) for eight cows were available. In this table the figures for jugular blood have been corrected for the influence of salivary secretion.

Table II.

No. of cow	Lactating or non-lactating	*Iron (mg. Fe per 100 cc. of blood)			Urea (mg. N per 100 cc. of blood)			Amino-acids (mg. N per 100 cc. of blood)			
		Jugular	Arterial	Mammary	Jugular	Arterial	Mammary	Jugular	Jugular (corr.)	Arterial	Mammary
1	Lactating	37.04	35.06	35.06	10.08	8.68	9.24	7.554	7.148	7.382	6.754
2	"	38.75	37.71	37.33	9.59	9.87	10.99	8.549	8.235	9.438	8.170
3	"	40.86	39.29	39.50	12.18	12.25	12.60	7.766	7.508	7.126	6.062
4	"	36.69	35.14	34.26	10.36	10.57	10.85	6.241	5.977	5.475	4.813
5	Non-lactating	39.31	36.28	36.81	6.58	6.86	7.21	6.921	6.480	6.591	6.993
6	"	34.74	—	32.94	11.41	11.03	11.34	7.653	7.257	7.848	7.653
7	"	34.23	32.56	33.46	8.61	9.31	8.96	7.147	6.985	7.204	7.384
8	"	37.28	34.60	34.89	11.74	11.20	11.74	6.413	6.003	6.101	6.388

* Iron determinations taken from Blackwood and Stirling [1932, 3].

In Table III the differences in the amino-acid-nitrogen contents of the blood samples are expressed as percentages of the arterial value for each animal. Table IV gives the results of a number of cases in which corpuscular volumes were available for the calculation of the amino-acid-nitrogen content of the corpuscles.

Table III.

No. of cow	Lactating or non-lactating	Differences in amino-acid-N content (as percentages arterial amino-acid-N)		
		Arterial-jugular	Arterial-mammary	Jugular-mammary
1	Lactating	3.16	8.49	5.33
2	"	12.75	13.44	0.69
3	"	— 5.36	14.93	20.29
4	"	— 9.17	12.09	21.25
5	Non-lactating	1.68	— 6.1	— 7.78
6	"	7.51	2.49	— 5.05
7	"	3.04	— 2.5	— 5.54
8	"	1.61	— 4.70	— 6.31

Table IV.

No. of cow	Lactating or non-lactating	Corpuscular volume		Amino-acid-N (blood) mg. N per 100 cc. of blood		Amino-acid-N (plasma) mg. N per 100 cc. of plasma		Amino-acid-N (corpuscles) mg. N per 100 cc. of corpuscles	
		Jugular	Mammary	Jugular	Mammary	Jugular	Mammary	Jugular	Mammary
1	Lactating	33.5	30.5	7.554	6.754	4.248	3.380	14.11	14.44
2	"	37.0	36.0	7.766	6.062	4.808	2.242	12.80	12.85
3	"	41.0	39.0	8.549	8.170	4.687	4.206	14.10	14.37
4	"	39.0	37.0	6.241	4.813	3.224	1.419	10.96	10.59
5	"	40.0	39.0	5.479	4.785	2.357	1.857	10.16	9.37
6	"	43.0	42.5	8.314	7.863	3.714	2.601	14.41	14.71
7	Non-lactating	41.0	38.5	6.921	6.993	3.949	4.094	11.19	11.62
8	"	36.5	35.5	7.653	7.731	4.584	4.658	12.99	13.31
9	"	32.5	30.0	7.147	7.384	4.790	4.874	12.04	13.24
10	"	38.3	34.8	6.413	6.388	4.056	3.978	10.21	11.00
11	"	41.6	37.5	5.922	5.934	3.917	3.948	8.736	9.145
12	"	40.0	39.0	5.949	5.934	3.819	3.857	9.154	9.202

DISCUSSION.

Cary [1920] gives values for the amino-acid-nitrogen content of the jugular blood of cows which range from 3.99 to 5.34 mg. N per 100 cc. These are lower than the values quoted by Bock [1917] and Okada [1918] who give 7 mg. N per 100 cc. as an average. Allardyce *et al.* [1930] give 5–8 mg. N per 100 cc. of

blood, a range which is in close agreement with the values obtained in the present work (5.8–8.5 mg.). Hayden and Fish [1928] give 5.30–18.0 mg. N per 100 cc. of blood as the range of urea-nitrogen encountered by them in the analysis of the blood of dairy cows, while Allardyce *et al.* give 12–20 mg. N. The urea-nitrogen values obtained in this series range from 5.4 to 12.6 mg.

As will be seen from Table I the differences between the amino-acid-nitrogen contents of jugular and mammary blood are generally very considerable. In this respect the results are in agreement with those of Cary.

From Tables II and III it is seen that in the non-lactating animals the amino-acid values of jugular blood are slightly lower than those of the arterial blood, a result which might be expected to arise from the utilisation of amino-acids by the tissues of the head and neck. The mammary venous blood of the four non-lactating animals examined is, however, slightly richer in amino-acid-nitrogen than arterial blood. This fact can only be explained on the assumption that amino-acids are returned to the blood during its passage through the inactive gland. The following explanation may be advanced to account for this finding. In dairy cows there is usually a period of three months between the end of one lactation and the beginning of the next. At the end of the lactation period part of the udder tissue which, in high milking breeds, may be very extensive, is resorbed. After a period of rest the animal again begins to proliferate mammary tissue in preparation for the next lactation. As the observations on non-lactating animals have been carried out soon after the end of the lactation period, it may be that the observed differences in amino-acid content between arterial and mammary venous blood are due to the breakdown of tissue-protein in the udder, and the gradual re-entry of the amino-acids so formed into the circulation.

With lactating animals such a consideration is, of course, absent.

It will be seen from Table III that in each of the four lactating animals examined the amino-acid-nitrogen content of the mammary blood is markedly and consistently lower than that of the arterial blood. Only a small proportion of this absorbed nitrogen can be accounted for by the renewal of glandular tissue, so that the theory that milk-protein is synthesised from the free amino-acids of the blood appears to be firmly established.

The assumption that the amino-acid-nitrogen content of jugular blood is the same as that of arterial blood is not supported by the data now presented. In all the non-lactating animals, and in two of the lactating animals, it is lower, while in the remaining two lactating animals it is higher. No explanation can be advanced to interpret this latter fact, but it is quite clear that the amino-acid-nitrogen content of jugular blood cannot be taken as an accurate representation of that of arterial blood.

From the qualitative standpoint this may not be important, but where attempts are made to interpret the data quantitatively (as has, for instance, been done by Meigs [1922]), the observed differences between arterial and jugular bloods will invalidate any conclusions.

In this connection it seems appropriate to consider some data on the relative absorption of sugar and amino-acids from the blood-stream. In a previous paper it has been shown that different animals absorb different amounts of sugar from the blood-stream. In this paper it is shown that different animals absorb different amounts of amino-acid-nitrogen. It is of value therefore to discover whether the ratio glucose absorbed/amino-acid-N absorbed is the same for different animals. Differences in the sugar and amino-acid-nitrogen contents of the arterial and mammary venous blood of four lactating cows are available (Table V).

Table V.

No. of cow	Sugar absorbed from 100 cc. blood arterial-mammary mg. glucose	Amino-acid-N absorbed from 100 cc. blood arterial-mammary mg. N	Sugar
			Nitrogen
1	5	0.628	7.9
2	16	1.268	12.6
3	8	1.064	7.5
4	10	0.662	15.0

From the above data it is seen that the ratio is not constant for different animals. It may then be expected that the sugar/nitrogen ratio in the milk from different animals will show variations which can be correlated with the above. That this is the case is shown in the figures 8.8, 9.5, 13.4, 15.6 obtained for the sugar/nitrogen ratios of the milk of four lactating cows taken at random from the same herd.

It has been noted above that although the amino-acid-nitrogen content of jugular blood cannot be taken to represent that of arterial blood in a strictly quantitative fashion, significant differences between jugular and mammary venous bloods may be accepted as indications of absorption. The procedure adopted in Table IV, where a comparison is made of the corpuscular amino-acid-nitrogen contents of jugular and mammary bloods, may justifiably be used to determine qualitatively whether the amino-acids of the corpuscles play any part in secretion. The fact that salivary secretion does not appear to affect the corpuscles in any way allows figures for jugular and mammary corpuscles to be compared directly without correction.

It will be seen that the ratio of amino-acid-nitrogen in the corpuscles to amino-acid-nitrogen in the plasma is approximately 3 to 1. No previous data on this point have been published, although Martens [1928] gives the figure of 3 or 4 to 1 for the human subject. In general, although relatively large differences are shown by the whole bloods and by the plasmas of lactating animals, only very small differences are shown by the corpuscles. In most cases, indeed, the amino-acid-nitrogen content of the mammary corpuscles is even higher than that of the jugular corpuscles. If the differences between the amino-acid contents of corpuscles and of plasma are due to indiffusibility (the only explanation which appears valid), we should expect *a priori* that

corpuscular amino-acids would normally play little or no part in direct exchanges between the blood and the mammary gland. That this is the case is amply borne out by the data presented.

SUMMARY.

1. In non-lactating cows the amino-acid-nitrogen content of the jugular blood is lower than that of arterial blood. The amino-acid-nitrogen content of mammary venous blood appears to be slightly higher than that of arterial blood, a fact which may be explained by the resorption of mammary tissue proteins during the post-lactation period.

2. In lactating cows the amino-acid-nitrogen content of jugular blood also differs from that of arterial blood. The amino-acid-nitrogen content of mammary venous blood is however always significantly lower than that of either jugular or arterial blood. This fact confirms the view that amino-acids are the precursors of milk protein.

3. It has been shown that the ratio of sugar to nitrogen absorbed by the mammary gland is different in different animals. The range of its variation is identical with that of the ratio sugar to nitrogen found in milk.

4. The ratio of corpuscular amino-acid-nitrogen to plasma amino-acid-nitrogen is approximately 3 : 1 for the dairy cow.

5. Evidence is brought forward which indicates that the amino-acids of the corpuscles play no part in the exchanges between the blood and the mammary gland.

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