LXIII. A REDETERMINATION OF THE TITRATION DISSOCIATION CONSTANTS OF ARGININE AND HISTIDINE WITH A DEMONSTRATION OF THE ZWITTERION CONSTITUTION OF THESE MOLECULES.

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(*Received February 28th, 1930.*)

We were prompted to undertake the present redetermination by the consideration that certain values recently put forward are in serious conflict with those previously accepted. (1) In the first place, Simms [1928] has given constants for arginine, and, although he presents no experimental evidence in their support, and notwithstanding the fact that they diverge widely from earlier values, he nevertheless uses them as the basis for postulating that arginine exists in protein in the form of some unknown precursor, termed "prearginine." We do not propose to discuss here the validity of Simms's assumption that a knowledge of the p_k value of a given group in an amino-acid enables one as yet to fix its value when it is combined in a complex protein molecule. Our purpose is merely to record results which confirm the earlier constants as opposed to those used by Simms. (2) In the case of histidine, Hirsch's [1924] values and curves differ from Harris's [1923, 1] conclusions in showing only one acid and one basic group instead of one acid and two basic groups. Further, the magnitude of the two p_k constants given by Hirsch are not in particularly good agreement with the earlier values. In our redetermination we have confirmed the earlier conclusions, both as to the orders of magnitude, and also the number, of the constants. The issue is of some interest because several workers have made use of Harris's description of tyrosine and histidine as diacid-monobase and dibase-monoacid ampholytes, respectively, to account for the acid- and base-binding power of proteins [*e.g.* Greenberg and Schmidt, 1924; Cohn and Berggren, 1925].

While carrying out the redeterminations we also proceeded to obtain experimental evidence of the zwitterion constitution of arginine and histidine.

I. Arginine.

From conductivity measurements of arginine salts, Kanitz [1906] first attempted to calculate the ionisation constants; but, "as pointed out by Harris [1923, 1], Kanitz's tabulation of his results contains misprints which completely distort some of his actual conclusions" [Hunter and Borsook, 1924], two out of the three constants being incorrectly set out. Harris [1923, 1], from a revision of the conductivity data of Kanitz, as well as his

own colorimetric, electrometric and p_H-end-point determinations, described the predicted titration curve, consisting of two well separated components having mid-points at p_H 2·3 and 9, plus a third value in an inaccessibly alkaline zone approaching p_H 13–14; later a direct titration curve led him to the very accurately calculated value of 2·17 for the first component [Harris, 1925][1]. Hunter and Borsook [1924] and also Hirsch [1924] obtained titration curves giving p_k values very similar to the above, viz. 2·17, 9·10 and 12·84; and 2·07, 9·18, —, respectively. Simms [1928], on the other hand, has cited values of 2·29, 9·64, and 8·15, the last being described as relating to the =NH group.

Experimental.

1. *Titration in water.*

Several specimens of free arginine base, from two different sources, were used and the technique employed was that described in earlier papers [Harris, 1923, 1, 2; 1925], which has since been generally adopted for determining dissociation constants in place of conductivity and other methods. The arginine was dissolved in water at approximately 0·1 *N* dilution and placed in the small specimen tube which was employed as titration vessel, and in which an atmosphere of hydrogen sufficed to prevent any absorption of CO_2 in the alkaline zone of the determination. The vessel was placed in a large box electrically regulated to a constant temperature, p_H measurements being made by use of the following: hydrogen electrode of the Hildebrand-Cole pattern, saturated calomel half-cell, agar-KCl bridge, and Cambridge and Paul portable potentiometer. As absolute standard 0·1 *N* hydrochloric acid was adopted, for which a p_H value of 1·07 was assumed; and the calomel cell was standardised against it. In order to diminish the magnitude of the blank correction in the titrations, and hence increase the accuracy of the determination [Harris, 1923, 1, p. 460], *N* HCl and NaOH were used rather than 0·1 *N*, with a microburette graduated in 0·01 cc.

From the p_H resulting after each addition of NaOH or HCl, p_k values were calculated by the Henderson-Hasselbalch approximation equations, as modified to allow for the blank correction for free NaOH or HCl [Harris, 1923, 1].

$$p_k = p_H - \log \frac{[Na] - [OH^-]/\gamma}{[\text{total amino-acid}] - ([Na] - [OH^-]/\gamma)}$$

$$p_k = p_H + \log \frac{[Cl] - [H^+]/\gamma}{[\text{total amino-acid}] - ([Cl] - [H^+]/\gamma)}.$$

Each value of γ, required in solving the above formula, was calculated on the basis of the total concentration of sodium (or chloride) present at the reading in question, and was arbitrarily assumed to be the same as for an equal concentration of NaOH (or KCl) in water alone—approximations giving results

[1] The approximate p_K 9 value was fixed by determining its p_H-end-point, which was found to be p_H 7 [Harris, 1923, 1, pp. 453, 482, 451] and also electrometrically [Harris, 1923, 2, p. 3300]; whilst the highly alkaline value was based on the observation that no measurable alkali was needed when titrating arginine to the standard end-point p_H 11·5, satisfactory for other amino-acids [Harris, 1923, 1, pp. 479].

sufficiently accurate for the present purpose [Harris, 1925]. These values of γ for NaOH and KCl were taken from the result of Harned [1925] and Noyes and McInnes [1920], respectively. If great precision is required in the very acid part of the curve, $p_H < 2{\cdot}2$, it would be more accurate to take somewhat higher values for γ, *i.e.* approximating more towards γ for HCl.

The experimental results and values derived therefrom are tabulated below.

Table I. *Titration of arginine with NaOH at 23°**.

0·1050 g. dissolved in 5·0 cc. of CO_2-free water and titrated with 1·08 *N* NaOH.

			(*d*)				
(*a*) E.M.F.	(*b*) p_H	(*c*) [H·]	cc. of 1·08 *N* NaOH added	Expressed as *N*/1 NaOH	(*e*) Total vol.	(*f*) [NaOH] $=d/e$	(*g*) γ corresponding with *f*
868·0	10·53	$0{\cdot}30 \times 10^{-10}$	0·000	0·000	5·00	—	—
898·5	11·05	$0{\cdot}89 \times 10^{-11}$	0·022	0·024	5·02	0·0046	0·92
926·0	11·52	$0{\cdot}30 \times 10^{-11}$	0·050	0·054	5·05	0·0107	0·92
946·0	11·85	$0{\cdot}14 \times 10^{-11}$	0·086	0·093	5·09	0·0183	0·88
955·0	12·00	$1{\cdot}00 \times 10^{-12}$	0·115	0·125	5·12	0·0244	0·88
963·0	12·14	$0{\cdot}73 \times 10^{-12}$	0·150	0·162	5·15	0·0315	0·86
979·0	12·40	$0{\cdot}40 \times 10^{-12}$	0·260	0·283	5·26	0·0538	0·82
987·5	12·56	$0{\cdot}28 \times 10^{-12}$	0·360	0·390	5·36	0·0728	0·78
994·0	12·66	$0{\cdot}22 \times 10^{-12}$	0·470	0·509	5·47	0·0931	0·77
1009·0	12·91	$0{\cdot}12 \times 10^{-12}$	0·850	0·920	5·85	0·157	0·75

(*h*) [OH′] $=K_w/[H^\cdot]$	(*i*) [Free NaOH] $=[OH']/\gamma$	(*j*) [Combined NaOH] $=f-i$	(*k*) cc. of combined NaOH, 1·0 *N* $=j \times e$	(*l*) Equivs. of combined NaOH	(*m*) log $\alpha/(1-\alpha)$	(*n*) p_k
—	—	—	—	—	—	—
0·00124	0·00135	0·0033	0·0164	—	—	—
0·00367	0·00398	0·0067	0·0339	0·029	+1·51	13·03
0·00786	0·00893	0·0094	0·0479	0·053	+1·25	13·10
0·0110	0·0125	0·0119	0·0609	0·076	+1·09	13·09
0·0151	0·0176	0·0139	0·0712	0·092	+0·99	13·13
0·0275	0·0355	0·0183	0·096	0·125	+0·84	13·24
0·0393	0·0504	0·0224	0·120	0·176	+0·67	13·23
0·0500	0·0650	0·0281	0·154	0·230	+0·52	13·18
0·0916	0·122	0·035	0·209	0·322	+0·32	13·23

* In view of the fact that specimens of arginine base may contain variable amounts of water of hydration and possibly also traces of combined acid the following method was adopted to convert actual amounts of HCl or NaOH added into true equivalents of HCl or NaOH, *i.e.* equivalents corrected for normality of the arginine preparation. The p_H values (ordinates) were plotted against the HCl (or NaOH) actually added (abscissae), and the scale for the abscissae was then inserted as equivalents, so as to show exactly one unit between the two well marked points of inflection which occur at about p_H 6 and 11, representing monohydrochloride and free base respectively. The values on this scale of equivalents are used in the above table.

The specimen of arginine on which the above results were obtained gave on combustion: N = 30·2 % (by titration, 4 × amino-N = 31·6 %; theory for $C_6H_{14}O_2N_4$ = 32·18).

A duplicate titration curve was determined upon a second specimen of arginine, derived from the same source (Hoffmann la Roche), and gave closely concordant values (not shown).

A specimen of "arginine" obtained from Pfanstiehl on the other hand, although described as of 100 % purity, required only 81·3 % of the theoretical amount of HCl to titrate one group; gave irregular p_k curves; and on analysis showed C = 38·47 %, H = 7·90 %, N = 26·57 % (theoretical for $C_6H_{14}O_2N_4$: C = 41·34 %, H = 8·10 %, N = 32·18 %). Its titration values are therefore neglected.

Table II. *Titration of arginine with HCl at 23°.*

0·1050 g. dissolved in 5·0 cc. of CO_2-free water and titrated with 1·0 *N* HCl.

(*a*) E.M.F.	(*b*) p_H	(*c*) $[H^+]$	(*d*) cc. of *N* HCl added	(*e*) Total vol.	(*f*) $[HCl]=d/e$	(*g*) γ corresponding with f	(*i*) [HCl free] $=[H^+]/\gamma$	(*j*) [HCl combined] $=f-i$	(*k*) cc. of combined HCl, $1{\cdot}0N=j\times e$	(*l*) Equivs. of combined HCl	(*m*) $\log a/(1-a)$	(*n*) p_k
868·0	10·52	—	0·000	—	—	—	—	—	—	0·028	—	—
852·0	10·25	—	0·020	—	—	—	—	—	—	0·060	—	—
837·0	10·00	—	0·048	—	—	—	—	—	—	0·108	−0·91	9·09
826·0	9·82	—	0·080	—	—	—	—	—	—	0·162	−0·71	9·11
814·0	9·62	—	0·126	—	—	—	—	—	—	0·240	−0·50	9·12
804·0	9·44	—	0·170	—	—	—	—	—	—	0·314	−0·34	9·10
794·0	9·27	—	0·226	—	—	—	—	—	—	0·406	−0·17	9·10
784·0	9·11	—	0·280	—	—	—	—	—	—	0·498	+0·00	9·11
772·5	8·91	—	0·345	—	—	—	—	—	—	0·590	+0·16	9·07
761·5	8·73	—	0·402	—	—	—	—	—	—	0·708	+0·38	9·11
749·5	8·59	—	0·454	—	—	—	—	—	—	0·790	+0·57	9·16
734·0	8·26	—	0·506	—	—	—	—	—	—	0·876	+0·84	9·10
725·5	8·12	—	0·526	—	—	—	—	—	—	0·916	+1·04	9·16
708·0	7·83	—	0·550	—	—	—	—	—	—	0·950	+1·28	9·11
670·0	7·18	—	0·574	—	—	—	—	—	—	0·990	—	—
620·0	6·33	—	0·582	—	—	—	—	—	—	1·006	—	—
475·5	3·89	0·00013	0·600	5·60	0·107	0·74	0·00017	0·107	0·599	0·032	—	—
448·5	3·43	0·00037	0·620	5·62	0·110	0·74	0·0005	0·109	0·615	0·052	—	—
427·0	3·07	0·00085	0·656	5·66	0·116	0·74	0·0012	0·115	0·651	0·118	−0·87	2·20
414·5	2·85	0·0014	0·695	5·70	0·122	0·73	0·0019	0·120	0·684	0·175	−0·67	2·18
402·0	2·64	0·0023	0·748	5·75	0·130	0·73	0·0032	0·127	0·730	0·256	−0·46	2·18
392·0	2·47	0·0034	0·800	5·80	0·138	0·73	0·0047	0·133	0·771	0·320	−0·33	2·14
380·5	2·28	0·0053	0·868	5·87	0·150	0·72	0·0074	0·143	0·839	0·440	−0·10	2·18
369·5	2·09	0·0081	0·950	5·95	0·160	0·72	0·0113	0·149	0·886	0·514	—	—
358·0	1·90	0·013	1·048	6·05	0·174	0·71	0·0183	0·156	0·944	0·622	—	—
347·0	1·72	0·019	1·155	6·16	0·188	0·71	0·0268	0·161	0·992	0·690	—	—

Table III. *Titration of arginine with NaOH at 17°.*

0·1050 g. dissolved in 5·0 cc. of CO_2-free water and titrated with 1·08 *N* NaOH.

(*a*) E.M.F.	(*b*) p_H	(*c*) $[H^+]$	(*d*) cc. of 1·08 *N* NaOH added	(*d*) Expressed as *N*/1 NaOH	(*e*) Total vol.	(*f*) [NaOH] $=d/e$	(*g*) γ corresponding with f	(*h*) $[OH']=K_w/[H^+]$	(*i*) [Free NaOH] $=[OH']/\gamma$	(*j*) [Combined NaOH] $=f/i$	(*k*) cc. of combined NaOH, $1{\cdot}0\ N=j\times e$	(*l*) Equivs. of combined NaOH	(*m*) $\log a/(1-a)$	(*n*) p_k
904·0	11·28	$0{\cdot}53\times10^{-11}$	0·021	0·023	5·02	0·0045	0·92	0·0015	0·0016	0·0029	0·0014	—	—	—
933·0	11·80	$0{\cdot}16\times10^{-11}$	0·042	0·045	5·04	0·0090	0·92	0·0049	0·0054	0·0037	0·0018	—	—	—
950·0	12·10	$0{\cdot}80\times10^{-12}$	0·070	0·076	5·07	0·0149	0·88	0·0099	0·0112	0·0037	0·0018	—	—	—
960·5	12·27	$0{\cdot}54\times10^{-12}$	0·100	0·108	5·10	0·0212	0·88	0·0146	0·0166	0·0046	0·0024	—	—	—

Table IV. *Titration of arginine with HCl at 17°.*

0·1050 g. dissolved in 5·0 cc. of CO_2-free water and titrated with 1·0 *N* HCl.

(*a*) E.M.F.	(*b*) p_H	(*c*) [H·]	(*d*) cc. of *N* HCl added	(*e*) Total vol.	(*f*) [HCl]=*d*/*e*	(*g*) γ corresponding with *f*	(*i*) [HCl free] =[H·]/γ	(*j*) [HCl combined] =*f* − *i*	(*k*) cc. of combined HCl, 1·0*N* =*j* × *e*	(*l*) Equivs. of combined HCl	(*m*) log *a*/(1 − *a*)	(*n*) p_k
867·5	10·70	—	0·000	—	—	—	—	—	—	0·028	—	—
849·0	10·35	—	0·022	—	—	—	—	—	—	0·064	—	—
839·5	10·21	—	0·042	—	—	—	—	—	—	0·098	−0·96	9·25
829·5	10·04	—	0·068	—	—	—	—	—	—	0·142	−0·78	9·26
820·5	9·88	—	0·100	—	—	—	—	—	—	0·196	−0·61	9·27
811·0	9·72	—	0·140	—	—	—	—	—	—	0·264	−0·44	9·28
802·5	9·56	—	0·184	—	—	—	—	—	—	0·338	−0·29	9·27
793·0	9·43	—	0·238	—	—	—	—	—	—	0·430	−0·12	9·31
784·0	9·27	—	0·288	—	—	—	—	—	—	0·514	+0·02	9·29
773·0	9·09	—	0·350	—	—	—	—	—	—	0·618	+0·20	9·29
763·0	8·93	—	0·402	—	—	—	—	—	—	0·708	+0·38	9·31
752·0	8·75	—	0·450	—	—	—	—	—	—	0·788	+0·56	9·31
736·0	8·47	—	0·498	—	—	—	—	—	—	0·870	+0·83	9·30
726·0	8·30	—	0·522	—	—	—	—	—	—	0·910	+1·00	9·30
705·5	7·94	—	0·548	—	—	—	—	—	—	0·956	—	—
646·0	6·90	—	0·570	—	—	—	—	—	—	0·990	—	—
465·0	3·75	—	0·590	—	—	—	—	—	—	0·026	—	—
444·0	3·38	0·0004	0·608	5·61	0·1085	0·74	0·0006	0·1080	0·606	0·046	—	—
431·0	3·16	0·0007	0·632	5·63	0·1122	0·74	0·0009	0·1113	0·626	0·096	−0·97	2·19
409·5	2·78	0·0016	0·700	5·70	0·1228	0·73	0·0023	0·1205	0·686	0·198	−0·61	2·17
398·0	2·58	0·0026	0·750	5·75	0·1305	0·73	0·0036	0·1269	0·729	0·270	−0·43	2·15
390·0	2·44	0·0036	0·800	5·80	0·1380	0·73	0·0049	0·1331	0·772	0·344	−0·28	2·16
380·0	2·26	0·0055	0·868	5·87	0·1480	0·72	0·0076	0·1404	0·824	0·434	−0·12	2·14
370·5	2·08	0·0083	0·948	5·948	0·1595	0·72	0·0155	0·1440	0·858	0·490	—	—
360·5	1·92	0·0120	1·028	6·028	0·1705	0·71	0·0169	0·1536	0·912	0·584	—	—
550·0	1·74	0·0182	1·130	6·130	0·1844	0·71	0·0263	0·1581	0·976	0·680	—	—
344·5	1·64	0·0229	1·200	6·200	0·1935	0·70	0·0327	0·1608	0·996	0·726	—	—

2. *Formaldehyde titrations.*

Titration curves were also determined in the presence of formaldehyde (Fig. 1), for this technique enables one to determine which of the p_k values relate to the amino- and which to the carboxyl groups and hence in any given case to obtain direct evidence [Harris, 1928, 1929] for or against the zwitterion formula—for which only indirect evidence had previously been available. The method, already applied by Harris to certain other amino-acids, rests on the following considerations. When formaldehyde is added to a simple base, the production of any methylene derivative, in equilibrium with the base, results in a diminution of the apparent basic strength, which is shown by a fall in the p_k value of the titration curve. Titration curves of acids on the other hand are, in general, naturally not affected by formaldehyde. Now when we titrate amino-acids, peptides or the like in formaldehyde we find that we get a number of p_k values shifted (towards diminished basicity) equal to the number of amino-groups in the molecule; and virtually no shift occurs in those remaining p_k values corresponding with the carboxyl groups. For example, glutamic acid, or reduced glutathione [Pirie and Pinhey, 1929], show two stationary and one shifted p_k values, lysine one stationary and two shifted, glycine one stationary and one shifted, and so on. The shifted p_k values in the amino-acid must clearly relate to the amino-groups for the following reasons. (1) In general, with mixtures of simple acids and bases of known p_k values it is always the basic and not the acid groups which are so shifted. (2) In ampholytes again, as just indicated, the number of p_k values shifted is equal to the number of amino-groups in the molecule and the number not shifted to the carboxyls. (3) If the contrary view were taken (*i.e.* that the shifted curve related to a carboxyl group and the unchanged to an amino-group) it would mean that formaldehyde causes no change in the measurable real basic strength, a conclusion difficult to reconcile with the fact that the formaldehyde is added (in Sørensen titrations) with the express purpose of weakening this basicity; it would also mean that there is an immense (*e.g.* 100,000 fold) increase in its *true* acid strength (*i.e.* in K_A, as opposed to k_a, the constant for an apparent acid group, as used in Harris's early papers where this effect of formaldehyde was first described),—a ridiculous supposition, most strikingly so in the case of molecules like polypeptides where the distance of the amino- from the carboxyl group would preclude the possibility that any slight change in the former would appreciably influence the strength of the latter. (4) Lastly it may be added that in its formaldehyde titration curve glycine behaves in a manner exactly parallel with ammonium acetate (the analogue of the zwitterion or "internal ammonium salt") and just contrary to a weak acid-weak base mixture (the analogue of the classical amino-acid formula).

In the case of arginine the p_k at 9·1 is due to an amino-group and that at 2·2 to a carboxyl group, for we found that the former p_k was shifted by formaldehyde in the direction of diminished basicity and the latter remained virtually

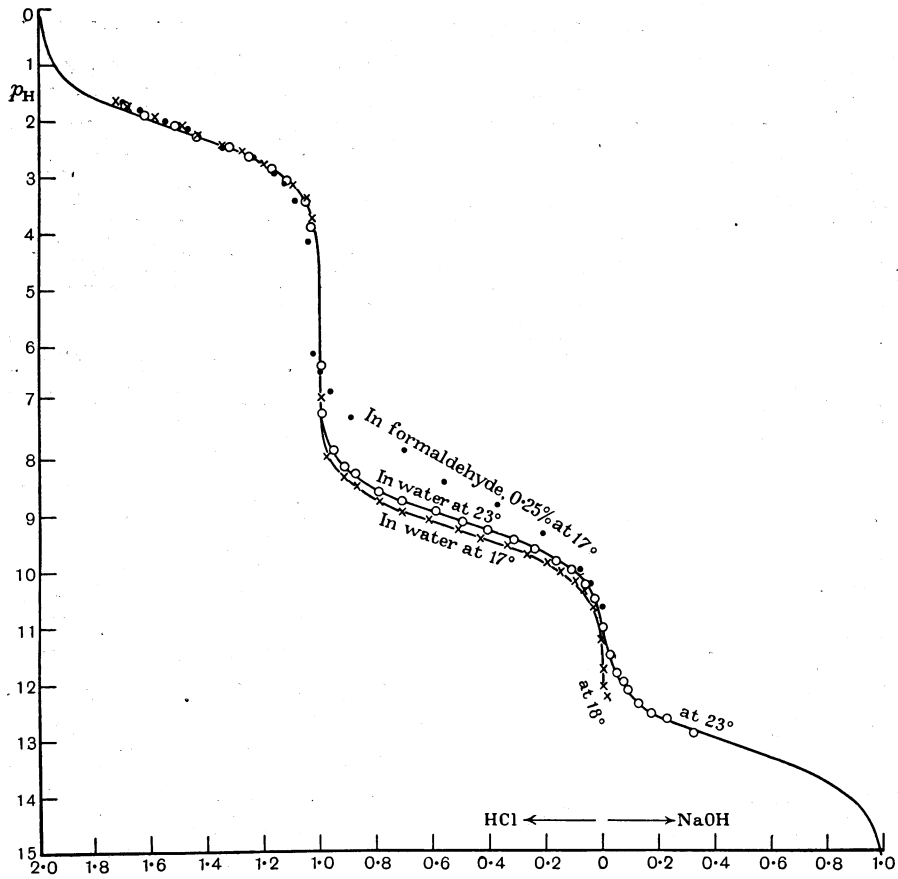

Fig. 1. Acid-base combination curve of arginine.

○ Experimental at 23° in water. × Experimental at 17° in water.

—— Theoretical curve drawn from $p_{k_1}=2{\cdot}18$, $p_{k_2}=9{\cdot}09$, $p_{k_3}=13{\cdot}2$ at 23°; or $p_{k_1}=2{\cdot}17$, $p_{k_2}=9{\cdot}29$, $p_{k_3}>13{\cdot}2$, at 17°. (The temperature shift in p_{k_2} corresponds with the change in the value of p_{K_w} and is assigned to a basic (amino) group with a small temperature coefficient for its K_B, while p_{k_1} is assigned to the carboxyl group with a K_A having similarly a relatively small temperature coefficient.

• • • Titration in formaldehyde (0·25 %) at 17°. With formaldehyde there is no appreciable shift in the p_{k_1} curve but considerable upward shift in the p_{k_2} curve (increasing with further additions of formaldehyde). It is therefore concluded that the latter relates to a basic (amino) group, the basicity of which is weakened in presence of formaldehyde; and the former to the carboxyl group, since it is found in general that the true acidity of carboxyl and similar groups is not appreciably affected by HCHO.

unchanged. The p_k values 9·1 and 2·2 are of the same order as those found for the amino- and carboxyl groups respectively in other α-amino-acids, and this of course is an additional argument in favour of our assignment. One concludes therefore that the p_k at 13–14 is due to the very strongly basic guanidine group, an assumption which is further confirmed by a direct titration curve which we have carried out on free guanidine base itself, from which we find that its dissociation constant is no less in magnitude but in fact somewhat larger even than that which we have assigned for the combined guanidine group of arginine.

3. *Magnitude of* p_k *temperature shifts.*

We have obtained yet further evidence supporting the above assignment, along somewhat novel lines, as follows. When titrations with the hydrogen electrode are carried out at several slightly differing temperatures, that curve which relates to the carboxyl group in the amino-acid (*e.g.* in glycine) shows no appreciable shift; that is, the p_k value has a small temperature coefficient. But, on the other hand, the titration curve for the amino-group shows a considerable shift with temperature, the magnitude of the shift depending mainly on the comparatively large change in the value of p_{K_W}[1], which of course enters into the hydrogen ion equation for a base, but not an acid; hence the magnitude of the real basic constant, p_{K_B}, calculated by subtracting p_k (*i.e.* "$p_{k_{AB}}$") from the value for p_{K_W} at the temperature in question, is, like the real acid constant, little affected by small temperature changes.

In general, the combination curve of a basic group will show less change with temperature when plotted on the OH than on the H scale, while that of an acid group will show less change with temperature when plotted on the H than on the OH scale; provided always that the temperature coefficient for the p_{K_A} (or p_{K_B}) is small compared with that for p_{K_W}. The reverse would be true were the temperature coefficients greater than that for p_{K_W}.

$$\text{For a base} \quad p_H = p_{K_W} - p_{K_B} - \log \frac{\alpha}{1-\alpha} \qquad \ldots\ldots 1,$$

$$\text{or} \quad p_{OH} = p_{K_B} + \log \frac{\alpha}{1-\alpha} \qquad \ldots\ldots 2.$$

$$\text{For an acid} \quad p_H = p_{K_A} + \log \frac{\alpha}{1-\alpha} \qquad \ldots\ldots 1,$$

$$\text{or} \quad p_{OH} = p_{K_W} - p_{K_A} - \log \frac{\alpha}{1-\alpha} \qquad \ldots\ldots 2.$$

That is, p_{K_W} with its appreciable temperature coefficient is involved in Equation 1, but not 2 for bases, and in Equation 2 but not 1 for acids. These relations between titration curve temperature shifts and temperature coefficient of dissociation constants will be discussed in detail in a later paper.

[1] For the purpose of the present treatment we are adopting the classical, pre-Brønsted formulation of base-acid equilibrium.

In the case of arginine the first HCl curve is shifted just as is the NaOH curve with glycine; but the second HCl curve is not appreciably shifted. We assume therefore that the first HCl curve (p_{k_2}) relates to a basic group. Its p_k value, if expressed as a p_{K_A} constant, would show a relatively large shift with temperature; whereas expressed as a p_{K_B} constant ($p_{K_B} = p_{K_W} - p_k$) it shows only a relatively negligible temperature shift. On the other hand, the value p_{k_1} expressed as an acid constant shows little change with temperature: we accordingly assume, in further confirmation of the earlier evidence given above that it relates to an acid group. In the case of p_{k_3} there is a significant increase with lowering of temperature, so that we have here again additional evidence for relating it to a basic group.

Table V. *Zwitterion constants for arginine.*

	Temp.	p_k	p_{K_A}	p_{K_B} ($=p_{K_W}-p_k$)
p_{k_1}	17°	2·17	2·17	—
	23°	2·18	2·18	—
p_{k_2}	17°	9·29	—	4·88 (=14·17 − 9·29)
	23°	9·09	—	4·87 (=13·96 − 9·09)

Our conclusions may be summarised and compared with Simms's, as follows.

Table VI. p_k *values*[1] *of arginine.*

	Birch and Harris (concentration = 0·1 *N*)		Simms
	temp. = 23°	temp. = 17°	
Guandine group	13·2	>13·2	8·15
α-Amino-group	9·09	9·29	9·64
Carboxyl group	2·18	2·17	2·29

II. Histidine.

Similar methods were applied to histidine, and details and results are appended below.

It will be noticed that the titration curve includes a second group titrating with acid at p_k 1·8, not shown by Hirsch.

Since this work was concluded Schmidt *et al.* [1929] have published values for histidine which are in excellent agreement with our figures, although these authors express the NaOH and second HCl curves as k_a and k_b constants respectively while we have definitely established them as K_B and K_A constants (from formaldehyde titrations, and a comparison of the magnitude of the temperature shift; see Fig. 2). p_{k_2} (first HCl curve) remains a basic group, and no doubt relates to the iminazole group.

[1] k is used throughout for the apparent or titration constant, and is not necessarily exactly identical with the true dissociation constant of the formal classical definition. K_A and K_B denote the titration constants expressed in the zwitterion mode, or the constants of true acids and bases: k_a and k_b denote the constants of apparent acids and bases.

Table VII. *Titration of histidine monohydrochloride hydrate with NaOH at 23°.*

Hofmann la Roche specimen: 0·1047 g. dissolved in 5 cc. of CO_2-free water, to make decimolar solution, and titrated with 1·08 *N* NaOH.

E.M.F.	p_H	cc. of NaOH, 1·08 *N*	NaOH in equivalents	$\log \alpha/(1-\alpha)$	p_k
477·0	3·94	0·000	0·000	0·00	—
527·5	4·80	0·028	0·060	+1·19	5·99
545·0	5·10	0·048	0·104	+0·93	6·03
557·5	5·31	0·076	0·164	+0·71	6·02
568·0	5·49	0·108	0·234	+0·51	6·00
577·0	5·64	0·148	0·320	+0·33	5·97
587·0	5·81	0·190	0·410	+0·16	5·97
597·0	5·98	0·234	0·506	−0·01	5·97
600·0	6·03	0·256	0·554	−0·09	5·94
605·0	6·12	0·278	0·602	−0·18	5·94
616·0	6·30	0·324	0·700	−0·37	5·93
632·5	6·58	0·376	0·812	−0·63	5·95
642·0	6·75	0·400	0·864	—	—
649·0	6·88	0·422	0·912	—	—
666·5	7·16	0·447	0·966	—	—
685·0	7·48	0·470	0·016	—	—
700·0	7·74	0·488	0·058	+1·21	8·95
713·5	7·97	0·506	0·094	+0·98	8·95
725·5	8·17	0·532	0·150	+0·75	8·92
737·5	8·38	0·562	0·216	+0·56	8·94
746·0	8·52	0·592	0·280	+0·41	8·93
757·0	8·71	0·630	0·360	+0·25	8·96
768·5	8·91	0·678	0·464	+0·06	8·97
774·5	9·01	0·700	0·512	−0·02	8·99
780·0	9·11	0·728	0·572	−0·12	8·99
794·0	9·34	0·782	0·690	−0·35	8·99
809·0	9·60	0·832	0·798	−0·59	9·01
818·0	9·75	0·860	0·858	−0·77	8·98
835·0	10·12	0·892	0·926	—	—
853·0	10·34	0·918	0·980*	—	—
877·0	10·75	0·932	1·002*	—	—
894·0	11·04	0·944	1·026*	—	—

* Corrected for blank.

Table VIII. *Titration of histidine monohydrochloride hydrate with HCl at 23°.*

Hoffmann la Roche specimen: 0·1047 g. dissolved in 5 cc. of CO_2-free water, to make decimolar solution, and titrated with 1·0 *N* HCl.

(*a*) E.M.F.	(*b*) p_H	(*c*) $[H^+]$	(*d*) cc. of *N* HCl added	(*e*) Total vol.	(*f*) $[HCl]=d/e+0·1$	(*g*) γ corresponding with *f*
477·0	3·96	0·0001	0·000	5·00	0·1000	0·74
431·0	3·17	0·0007	0·024	5·02	0·1044	0·74
413·0	2·86	0·0014	0·048	5·05	0·1095	0·74
399·0	2·63	0·0023	0·078	5·08	0·1138	0·74
387·5	2·43	0·0040	0·116	5·12	0·1203	0·73
378·0	2·27	0·0054	0·162	5·16	0·1284	0·73
368·0	2·09	0·0081	0·224	5·22	0·1392	0·73
358·5	1·93	0·012	0·290	5·29	0·1495	0·72
350·0	1·79	0·016	0·358	5·36	0·1601	0·72

(*i*) $[HCl\ free]=[H^+]/\gamma$	(*j*) [HCl combined] $=f-i$	(*k*) cc. of combined HCl, $1·0N=j\times e$	(*l*) Equivs. of combined HCl	(*m*) $\log \alpha/(1-\alpha)$	(*n*) p_k
—	0·1000	0·500	0·000	—	—
0·0009	0·1035	0·520	0·040	−1·38	1·79
0·0019	0·1077	0·544	0·088	−1·02	1·84
0·0031	0·1107	0·564	0·128	−0·83	1·80
0·0055	0·1148	0·588	0·176	−0·67	1·76
0·0074	0·1210	0·624	0·248	−0·48	1·79
0·0111	0·1281	0·669	0·338	−0·29	1·80
0·0167	0·1328	0·703	0·406	−0·17	1·76
0·0222	0·1379	0·739*	0·478	−0·04	1·75

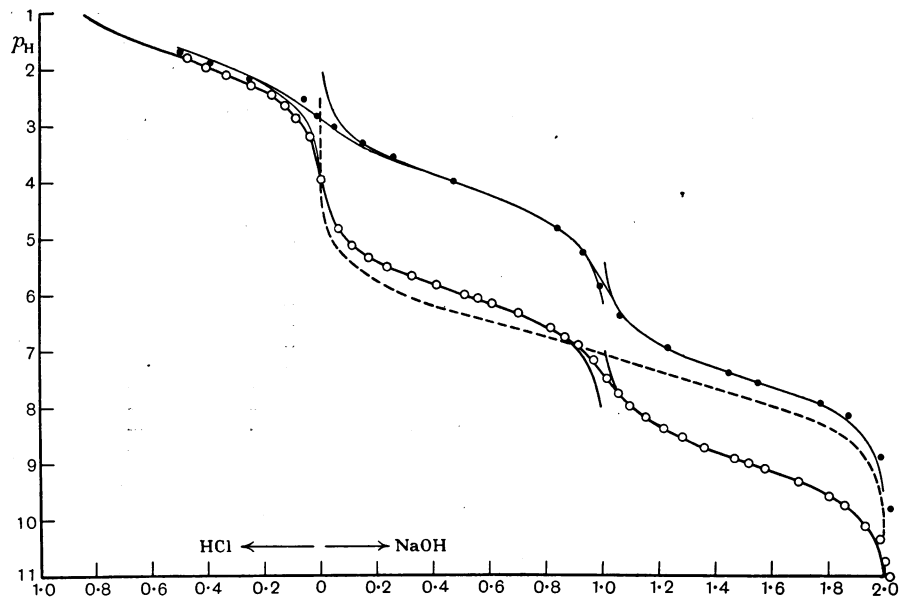

Fig. 2. Acid-base combination curve of histidine.

O Experimental (23°).

—— Theoretical curve for p_k values at 1·78, 5·97, 8·97. At a lower temperature, viz. 17°, p_{k_2} and p_{k_3} values were notably increased and therefore are assigned to basic groups. By analogy with all the α-amino-acids, the p_k at about 9—*i.e.* p_{k_3}—no doubt relates to the α-amino-group and hence the p_k at 6—*i.e.* p_{k_2}—relates to the iminazole group. Formaldehyde titration, again, confirms the assignment of p_{k_1} to the carboxyl and p_{k_2} and p_{k_3} to basic groups.

••• Formaldehyde titration readings, with theoretical p_k curves.

– – – Curve given by Hirsch showing only one acid and one basic group each possessing different p_k values from the above, and with no point of inflection at about p_H 7·5.

Table IX. p_k *values for histidine.*

Temp.	Calculated by Harris [1923, 1] from Kanitz's conductivity measurements	Hirsch [1924]	Simms [1928]	Schmidt, Appleman and Kirk [1929]	Birch and Harris
	25°	18°	25°?	25°	23°
p_{k_1}	1·7	—	1·46	1·82	1·78
p_{k_2}	5·8	6·32	6·06	6·01	5·97
p_{k_3}	8·7	7·74	9·41	9·17	8·97

Summary.

1. The p_k value 8·15 given by Simms for the guanidine group in arginine, on the basis of which he postulated the existence of a "prearginine" group in proteins, is widely divergent from a value, viz. p_k = *ca.* 13 to 14, previously deduced (1) by one of us from titration readings and Kanitz's conductivity data, and (2) electrometrically by Hunter and Borsook.

An accurate redetermination has given us the three p_K constants, 13, 9·09 and 2·18 ($t = 23°$, 0·1 N), in good agreement with earlier values.

It is shown, by Harris's formaldehyde titration technique, and by a new method involving measurement of the shift of $p_{K_{AB}}$ with temperature, that the first of these relates to the guanidine group, the second to an amino-group and the third to the carboxyl group. That is, the magnitude of the guanidine group's dissociation constant as determined by us is 100,000 times greater than that given by Simms.

2. Since the titration curve of histidine was originally reported by one of us to consist of three p_K components, whilst Hirsch described two only, a redetermination has been made which gives the three p_K values 8·95, 5·98 and 1·78 (at 23° and 0·1 N); the first relates to the amino-, the second to the iminazole and the third to the carboxyl group.

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