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THE SELECTIVE APPETITE FOR Na^+ SHOWN BY Na^+-DEFICIENT SHEEP

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In view of the importance of Na^+ equilibrium to normal function of higher species, an innate behaviour pattern promoting its intake in the face of deficiency could give an important survival advantage to the organism. Richter (1956) has shown that young rats, for whom their mother's milk has been the sole source of nourishment, consistently showed an active appetite for NaCl when confronted with it for the first time. He states that with mammals there is a universal liking for NaCl, and he considers this to be indicative of an inherited behaviour pattern which has conferred survival advantage. This primitive attraction for salt was modified by metabolic conditions, as is evidenced by the increased intake of NaCl solution which occurred when NaCl loss was caused by adrenalectomy.

Previous publications from this laboratory have described the preparation and maintenance of sheep which have a permanent unilateral parotid fistula (Denton, 1956, 1957*a*; Denton, Goding & Wright, 1959), and lose 1–4 l./day of alkaline parotid saliva containing 170–680 m-equiv of $NaHCO_3$. An animal prepared in this manner is an excellent subject for the study of physiological adaptations in the face of a controlled and precisely defined stress on the *milieu intérieur*. This paper reports experiments on the voluntary intake by sheep of Na-containing solutions, and the effect of Na^+ deficiency caused by loss of saliva on this voluntary intake.

METHODS

Twenty sheep of Merino or Merino cross-breeds were used. Six were ewes, the remainder were wethers. They were 1–7 years old. Apart from the normal sheep in Table 1 all had Wright-type permanent unilateral parotid fistulae and ipsilateral carotid artery loops. A number had contralateral carotid artery loops also. Three sheep, T.P. 1, T.P. 4, and T.P. 12, had the left adrenal gland transplanted to a combined carotid-artery–jugular-vein skin loop in the neck (McDonald, Goding & Wright, 1958) and the right adrenal gland removed.

Most of the sheep were purchased from sale yards, and their previous history was unknown in relation to the possibility of access to salt licks or to bore water containing salt. The animals were kept in stainless-steel metabolism cages which effectively separated excreta,

and permitted collection of uncontaminated specimens of urine, saliva and faeces (Denton, 1956). In experiments where electrolyte analyses were made on the saliva each day, the anterior compartment was washed daily with distilled water, and, in the case of external electrolyte balance studies, these washings were analysed also. Sheep were fed daily on 0·8–1 kg of a mixture of equal parts of oaten and lucerne chaff. This food was mixed so as to give large batches of uniform composition and was analysed at intervals. Thus the Na^+ intake from food was known and was approximately 100 m-equiv/day. For external electrolyte balance studies several food analyses were made in the course of the experiment, and the mixing was very thorough.

TABLE 1. Voluntary intake of various electrolyte-containing solutions and water by normal sheep, and sheep with a unilateral parotid fistula

Name of sheep	Experimental conditions	No. of days	Average voluntary intake (l./day)				Total volume (l./day)	Total m-equiv Na^+/day	Saliva volume (l./day)
			Water	NaCl	$NaHCO_3$	KCl			
Dido	Normal	16	0·91	0·51	0·28	0·06	1·76	332	—
	1 hr/day	9	—	0·08	0·04	—	—	50	—
	Normal	5	0·68	0·39	0·45	0·02	1·54	353	—
Lucy	Normal	10	1·40	0·83	0·88	0·65	3·76	718	—
Dolly	Normal	11	0·77	0·71	0·22	0·28	1·98	391	—
Edward	Normal	13	1·55	0·77	0·02	0·03	2·37	323	—
	1 hr/day	8	—	0·18	0	0	—	76	—
	Normal	5	1·24	1·11	0	0	2·35	466	—
Fred	Normal	11	1·81	0·88	0·74	(not offered)	3·43	680	—
	5–10 days post-op.	5	0·24	0·37	1·31	—	1·92	706	—
Fido	Normal	6	0·67	0·10	0·01	0·97	1·75	46	—
	17–25 days post-op.	8	0·95	0·48	0·27	0·99	2·69	305	0·81
Disraeli	Normal	15	1·01	0·51	0·26	0·38	2·16	323	—
	15–20 days post-op.	5	2·19	0·24	0·75	0·32	3·50	416	1·67
Charlie	Normal	8	1·99	0·11	0·01	0·01	2·12	50	—
	1–7 days post-op.	6	2·77	0·05	0·66	0·02	3·50	298	1·86
Fraser	Normal	8	1·71	0·14	0·08	0·08	2·01	92	—
	5–10 days post-op.	5	2·11	0·23	0·55	0·11	3·00	328	2·21
Cassie	Normal	13	0·67	0·42	0·24	0·38	1·71	277	—
	11–14 days post-op.	3	1·53	0·46	0·12	0·58	2·69	244	1·49
	21–27 days post-op.	6	1·25	0·39	0·53	0·62	2·79	386	1·33
	10 months post-op.	4	—	—	—	—	—	443*	2·34

* $NaHCO_3$ only available.

Solutions. Analytical reagent standard chemicals were used to make the solutions of electrolyte offered the animals. The appropriate amount was weighed out, and dissolved in tap water. The solutions were offered to the animal in stainless-steel bins, 30 cm × 20 cm × 15 cm. Each cage had an opening 45 cm long and 30 cm high at the anterior end on each side and two bins containing fluid could be attached to the outside on each side. The front door of the cage had a hinged flap 38 cm wide and 21 cm high and this was raised and the food bin attached in this position. With experiments lasting some weeks when four fluids (H_2O and solutions of NaCl, $NaHCO_3$, and KCl) were offered to the animal each day, the four places on the cage were numbered, and the position of the fluids was varied randomly on the basis of tossing two coins. The same amount of fluid was placed in each tin, and usually the volume was 3–4 l. The evaporation from the tins under summer conditions was approximately 50–100 ml./day. For this reason, solutions which were untouched or partly

drunk, and had been made up to original volume by addition of stock solution were discarded every third day. Specimens were taken from the bins at intervals and analysed to confirm the accuracy of the concentration. In experiments where the animal had access to the solutions for 1 hr only each day evaporation did not have to be taken into account.

Methods of observing behaviour. In some experiments the animals had access to food and various solutions continuously. In others the animal had access to the solutions for 15–60 min only at a standard time on the experimental day. In these circumstances the observer sat about 8 ft. (2·4 m) from the cage and recorded the animal's sampling behaviour. About 15 min before the sampling experiment two large steel sheets were hung on the inner sides of the cage, so that the animal was unable to see or explore the solutions as they were placed in position. At zero time the guards were removed and any of the following acts were recorded as a sampling 'episode'—dipping the head into a tin, dipping the jaw in the solution, taking the solution in the mouth, or drinking the solution. The number of episodes during the period of access was recorded. Under some conditions behaviour could be recorded satisfactorily by a shorthand notation, but under others the rapidity of movement and sampling required that the observer quietly described proceedings into the microphone of a tape recorder. The typewritten record of the episodes was then completed for time sequence by running the original tape record with a stop-watch. In the experiments where rate of drinking was measured, the act was timed with a stop-watch and the container removed, weighed quickly, and returned to its position.

Sometimes it was of interest to determine the preference of a sheep for a particular solution without allowing it to drink enough to alter its state of balance. Two cups, 10 cm in diameter and 5 cm deep were set 2·5 cm apart on a small tray which had a handle 43 cm long. At zero time the front door of the cage was opened and the animal had access to 50 ml. of solution in each cup. In the appropriate circumstances the animal would smell or taste one solution and reject it whereas it avidly drank the other and licked the cup.

Saliva secretion rate was measured over short intervals by counting the drops of secretion as they fell from the dependent point of the Wright fistula. The sheep, P.F. 33, P.F. 48, Ned and T.P. 12, which were used for experiments involving detailed observation of sampling behaviour, had all been in the laboratory for 1–3 years and were thoroughly accustomed to having members of the laboratory in their immediate vicinity for many hours each day. They were confident and apparently quite undisturbed by any procedure involved in these experiments.

Chemical methods were those described by Denton (1956).

RESULTS

The voluntary intake of electrolyte containing fluids by normal sheep

This was observed in ten sheep. They were offered four fluids (water, NaCl and $NaHCO_3$ (420 m-equiv/l.) and KCl (130 m-equiv/l.)). The solutions were available all day, the position being varied randomly each day. The sheep were given their fixed ration at the same time each day, providing a Na intake of 50–100 m-equiv/day. Usually the animals were offered water in all four tins for 5–10 days before the observation period began.

Table 1 shows the voluntary intake of the ten normal sheep. All the animals drank some Na^+-containing fluid, and with one exception (Lucy) there was a preference for NaCl over $NaHCO_3$. The average daily Na intake in different sheep varied from 46 to 718 m-equiv. Sometimes, but not

invariably (Figs. 1 and 2), the main intake was in the first days of the period, the amount being reduced after initial drinking episodes on the first 1 or 2 days. Urinary analyses were made in some animals and showed a substantial Na excretion. In the case of Lucy Na excretion exceeded 1000 m-equiv on some days. With the exception of Fido and Lucy, little KCl solution was drunk. In two sheep, after the control period, the electrolyte solutions were made available to the sheep for 1 hr/day only. The intake observed was small.

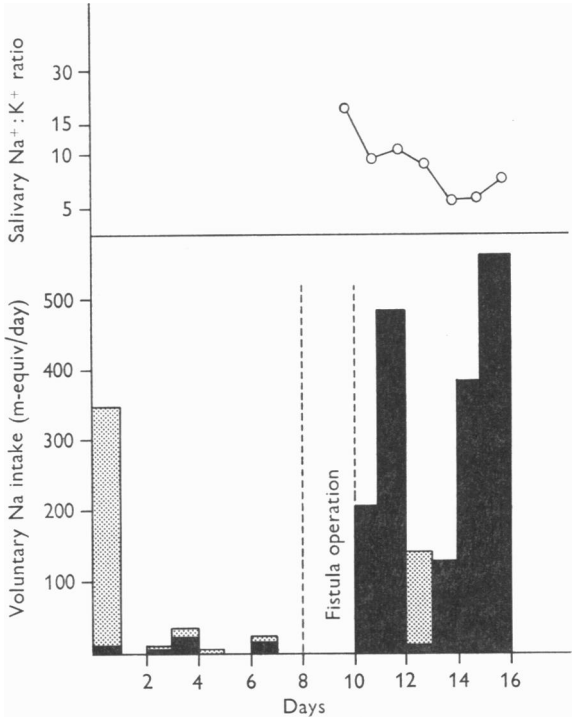

Fig. 1. Charlie, Merino wether. The voluntary daily intake of solutions of $NaHCO_3$ (■ 420 m-equiv/l.) and NaCl (▤ 420 m-equiv/l.) before and after the establishment of a permanent unilateral parotid fistula. The $Na^+:K^+$ ratio of the parotid saliva is shown also. KCl (140 m-equiv/l.) was offered concurrently but there was little or no intake (Table 1). These solutions and water were available all day.

With Charlie (Fig. 1), the behaviour before operation was examined in some detail. After an intake of NaCl when the solutions were offered first, the animal showed little interest in the electrolyte fluids. On one occasion during this pre-operative period the animal had no access to any fluid for 48 hr, and it was obviously thirsty at the end of the period. It was offered various solutions on the 'preference tester' (see Methods). On the first trial, $NaHCO_3$ and H_2O were offered. It encountered $NaHCO_3$, tasted it

and passed quickly to the other tray where it found water which it drank. Eleven tests were made and it drank H_2O whenever this was included but rejected NaCl, $NaHCO_3$ and KCl. Despite variation of position of various solutions offered, it appeared that after initial testing the sheep could identify the solutions on smell alone in the subsequent tests, and would, after moving its head across the tray, proceed immediately to water and drink. This animal began to drink $NaHCO_3$ on the first day after a parotid fistula operation, having shown no interest in it during the pre-operative period.

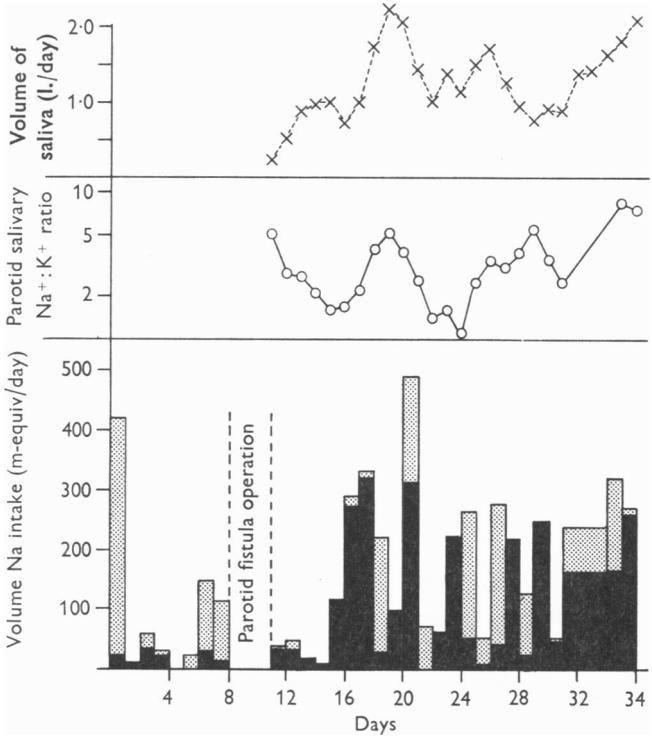

Fig. 2. Fraser, Merino wether. The voluntary daily intake of solutions of NaCl and $NaHCO_3$ before and after establishment of a permanent unilateral parotid fistula. The volume of saliva secreted each day, and the parotid salivary Na^+:K^+ ratio are shown also. The conditions and symbols similar to those for Fig. 1.

The voluntary intake by sheep with parotid fistula

In six sheep of Table 1 a parotid fistula operation was carried out, and the effect of saliva loss on the voluntary intake of the solutions was compared with the initial period. Figures 1 and 2 show the effect of the parotid fistula on the daily intake of two of the sheep. In no case was the daily saliva loss large (i.e. more than 2 l. = a Na^+ loss of *ca.* 300 m-equiv/

day). Where the pre-operative Na intake was small, there was a significant post-operative increase. In four of the six animals there was a clear post-operative preference for $NaHCO_3$ over NaCl—a more appropriate choice in the face of the $NaHCO_3$ loss from the fistula. With the two which did not show the preference the volume of fistula loss was small. Further observations recorded in sections below confirm a clear-cut preference for $NaHCO_3$ by sheep which lost a large volume (3–4·5 l. daily) from a parotid fistula. The salivary Na^+:K^+ ratio analyses in Figs. 1 and 2 show that the animal's voluntary intake was inadequate to prevent some degree of Na^+ deficiency during some periods; the normal Na^+:K^+ ratio of a Na^+-replete sheep is 20–30.

The effect of variation of concentration of $NaHCO_3$ solution on the amount of voluntary intake

This experiment was made on three sheep with parotid fistulae (P.F. 33, T.P. 1, and T.P. 4). All three secreted large volumes of saliva (3–4·5 l./day). They had been in the laboratory for 2–4 years and until 2 months before this experiment were maintained in normal Na^+ balance by giving 600–830 m-equiv of $NaHCO_3$ dissolved in 1·5 l. of H_2O each day by rumen tube. One of them, P.F. 33, had experience of self-selection procedures during this time under conditions where the daily intrarumenal supplement was withheld. During the 2 months before the experiment the sheep had been offered 2 l./day of a $NaHCO_3$ solution (300 m-equiv/l.) instead of being given Na by rumen tube. They maintained themselves in good condition on this régime. In the experiment described here the sheep were given 0·8 kg/day of the usual diet (*ca.* 100 m-equiv Na/day) and had free access to water. They also had access to a large volume of $NaHCO_3$ solution, the concentration of which was varied at 3–10 day intervals. Allowing the Na^+ content of food, the net negative Na^+ balance caused by the fistula would have been 400–580 m-equiv/day.

Figure 3 shows the experiment on T.P. 4. Following the long period of access to $NaHCO_3$ at 300 m-equiv/l., T.P. 4 was offered 950 m-equiv $NaHCO_3$/l. for 2 days. It drank 0·7 and 0·44 l., i.e. 665 and 420 m-equiv Na. On the third day the concentration was changed to 119 m-equiv/l. and the animal drank 4·86 l. = 583 m-equiv of Na on this day. The average intake for the period of 10 days with this concentration was 535 m-equiv/l. and the adaptation to this large change of concentration was made during the first day it was offered. During the next 10 days the concentration was 238 m-equiv/l. and the average Na intake was 616 m-equiv. In this instance the adaptation to change was slower, the average intake for the first 3 days being 794 m-equiv/day. At 476 m-equiv $NaHCO_3$/l. the average daily intake of Na was 428 m-equiv, and when the

concentration was increased again to 952 m-equiv/l. the average daily Na intake was 350 m-equiv. In both instances the animal adjusted the volume drunk to the concentration on the first day of change. After a further period of 7 days with concentration at 476 m-equiv $NaHCO_3$/l., 476 m-equiv of $NaHCO_3$ was added each day to the sheep's food. As Fig. 3 shows, it drank practically no $NaHCO_3$ solution after the first day.

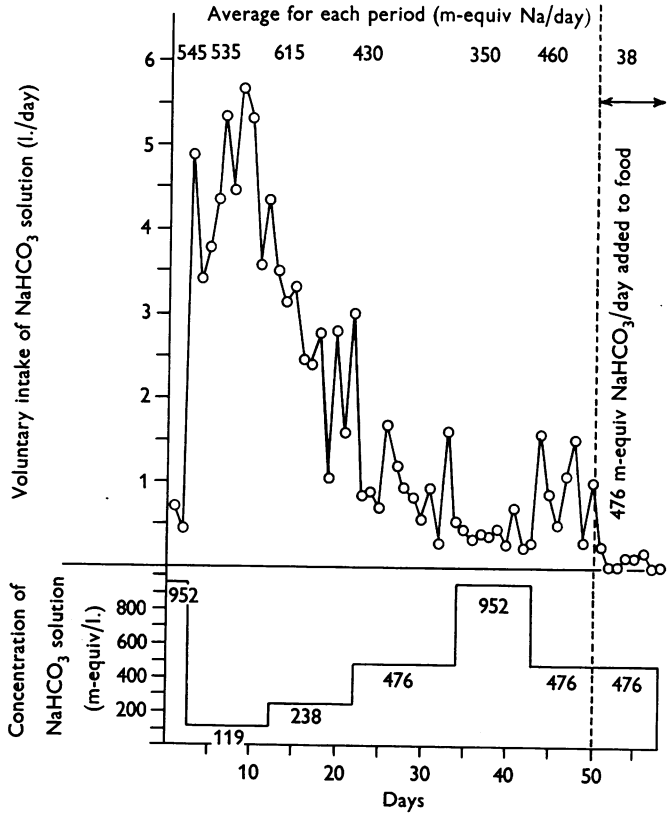

Fig. 3. T.P. 4, Merino wether. The volume drunk each day of a solution of $NaHCO_3$, the concentration of which was varied as shown in the lower section of the figure. Water was available continuously. The average intake (m-equiv Na/day) for each period is shown also at the top of the figure. After 50 days, 476 m-equiv of $NaHCO_3$/day was added to the food.

Table 2 summarizes the experiments on two other sheep. P.F. 33 was able to adjust intake to change of concentration but the adaptation was not made always on the first day of change of concentration. With T.P. 1, in two periods the intake exceeded considerably the daily loss of Na^+ from the fistula.

Summarizing, this series of experiments showed that the animals drank

voluntarily an amount of $NaHCO_3$ solution which approximated to the loss each day from the fistula. They were able to do this in the face of variation in concentration of solution offered and, in many instances, they adapted to a change of concentration immediately (i.e. during the first day the change was made). It appeared as if the sheep were able to 'multiply volume by concentration', which provoked interesting questions concerning the exact sampling behaviour of the animals. The next section and a subsequent paper deal with some aspects of this question.

TABLE 2. Voluntary daily intake, by sheep with permanent unilateral parotid fistulae, of $NaHCO_3$ when offered in different concentrations

Sheep	No. of days offered	$NaHCO_3$ concn. (m-equiv/l.)	Average volume drunk (l./day)	Average Na intake (m-equiv/day)
P.F. 33	9	238	2·13	507
	11	476	1·21	576
	11	952	0·70	666
	9	119	4·77	568
T.P. 1	5	952	1·13	1076
	7	119	4·46	531
	10	238	1·95	464
	12	476	0·77	366
	9	952	0·52	495
	17	476	1·96	932

Voluntary intake when Na solutions were available for one hour a day only

In this series of experiments water and food were available continuously to the animal. At the same time each day the solutions were presented for a period of 15–60 min only and, immediately following the test period, saliva and urine of the preceding 24 hr were taken for analysis. A feature of these experiments was the excitement and anticipatory behaviour of the sheep as the usual procedures indicating presentation of the Na^+ solutions were carried out. The degree of urgency shown by the animal was related to the degree of depletion, and the motor behaviour (foot stamping, etc.) was associated with a variety of visceral responses including salivary conditioned reflexes (Denton *et al.* 1960). During the period of access the number of episodes of sampling solutions, and the exact sequence of smelling and tasting them were recorded.

Experiments were made on P.F. 33, P.F. 40 and P.F. 43. Four randomly placed solutions (water, NaCl and $NaHCO_3$ (420 m-equiv/l.) and KCl (134 m-equiv/l.)) were available to the sheep for 1 hr only. Intake was observed during control periods when the sheep received 595–830 m-equiv/day of $NaHCO_3$ by intrarumenal tube and during Na^+ deficiency produced by withdrawal of this supplement. With P.F. 33 the voluntary intake during Na^+-replete stage was negligible (4 m-equiv Na during the

hour each day) and during the 7 days of depletion averaged 298 m-equiv of Na during the hour each day with a clear preference for $NaHCO_3$. The average number of sampling episodes involving the Na containers was 5 in the hour during the control period, whereas during deficiency there were 30–40 episodes in the hour. However 75% of the voluntary intake occurred in the first 5 min. Following readministration of the Na supplement, voluntary intake and interest in the solutions declined precipitately and the animal sometimes did not sample any solutions until 8–27 min after the observation hour had begun. The results with P.F. 40, and P.F. 43 which had had no experience of self-selection procedure before the experiment, were similar except that the intake of P.F. 43 was entirely as NaCl.

A further experiment including an external electrolyte balance was made on P.F. 33, lasting 34 days during which the solutions were offered in four ways:

(1) For the first 7 days water, NaCl, $NaHCO_3$ and KCl solutions were available all day and the average voluntary Na^+ intake was 602 m-equiv/day. Allowing for the Na content of food (116 m-equiv/day), the total Na intake approximated Na output. The salivary Na loss was 676 m-equiv/day, the urinary Na loss was 22 m-equiv/day and faecal loss was 3 m-equiv/day. The voluntary Na^+ intake was almost entirely of $NaHCO_3$.

(2) For the next 7 days, when the solutions were available each day for 1 hr, the average voluntary Na intake was 560 m-equiv in that hour. It was mainly $NaHCO_3$.

(3) In the next 8 days, when the solutions were available for 1 hr only on every second day, the average voluntary intake was 878 m-equiv in that hour. Approximately one third of this intake was as NaCl. The average amount by which Na loss in the 2 days exceeded food intake was 930 m-equiv.

(4) In the final period of 6 days, when the solutions were available for 1 hr on every 3rd day, average intake was 1137 m-equiv in that hour. Equal amounts of NaCl and $NaHCO_3$ were drunk. The average amount by which Na loss had exceeded intake by this third day was 1070 m-equiv.

Voluntary Na^+ intake in relation to Na^+ balance when concentration of Na^+ solution was varied

Figure 4 shows another experiment on P.F. 33 under different conditions. Water was available continuously throughout the experiment. The animal was fed at the same time each day. Saliva and urine were collected immediately following the test period when $NaHCO_3$ solution was offered for 1 hr each day, but the concentration of the offered solution was different in periods *b*, *c*, *d*, *e* of Fig. 4.

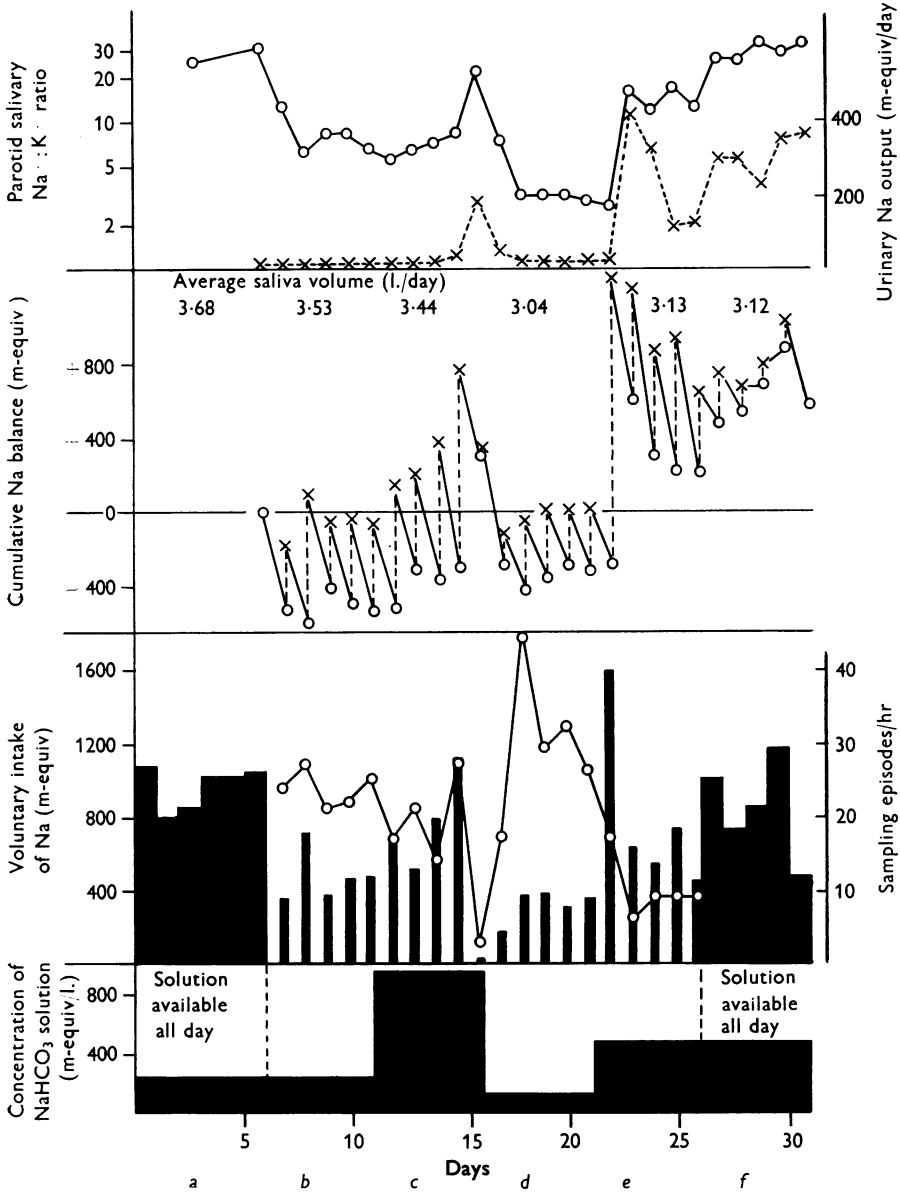

Fig. 4. P.F. 33, Merino cross-bred ewe. The top section records the parotid salivary $Na^+:K^+$ ratio (O — O) and the urinary Na excretion per day (× - - - ×). The second section records the cumulative Na^+ balance. The solid line (× — O) records the change of balance (m-equiv Na/day) and the interrupted line (O - - - ×) records the change in balance produced by voluntary intake during the hour when access to $NaHCO_3$ was permitted. The average salivary volume per day for each period of the experiment is shown also. The third section shows the voluntary intake of Na^+ when the animal had access all day, and (thin column) when there was access for 1 hr only. The number of sampling episodes during the hour of access (O — O) is shown also. The bottom section records the concentration of the $NaHCO_3$ solution which was offered. Water was available continuously.

For some months before the experiment the sheep had been maintained on a régime involving access to 2 l./day of a solution of $NaHCO_3$ 300 m-equiv/l. It usually drank the lot each day. During the 6-day control period (*a* in Fig. 4) the average intake of the solution continually offered, containing 238 m-equiv/l., was 970 m-equiv Na/day. The average saliva volume, 3·68 l./day, involved a daily loss of 680 m-equiv of Na. Hence the voluntary daily Na intake, which varied little, was considerably greater than fistula loss. Consistent with this, the salivary Na^+:K^+ ratio was normal. No urinary analyses were made, but as is indicated by the final section (*f* of Fig. 4) the excretion was probably 200–400 m-equiv/day.

During the period *b* of Fig. 4 the sheep had access to 238 m-equiv $NaHCO_3$/l. for 1 hr each day. The balance study chart is constructed with an assumption that, at the end of the period of continuous access to $NaHCO_3$, the animal was in Na^+ equilibrium. The solid line (×—○) indicates the change in external Na^+ balance during the 23 hr preceding the 1 hr test, and shows a decrease of Na content of the animal because the fistula loss greatly exceeded Na intake in food. The interrupted line (○– – –×) shows the change in Na^+ balance produced by the voluntary intake of Na during the hour period of access. The number of episodes of sampling the $NaHCO_3$ solution and water during the hour are shown on the voluntary Na intake section of the figure. The average intake in the hour each day of the 238 m-equiv $NaHCO_3$/l. was 476 m-equiv. As the cumulative Na^+ balance shows, the 23 hr negative Na^+ balance was usually over 500 m-equiv, so that the voluntary intake was about adequate to restore Na^+ equilibrium. Consistent with the fact that the external Na^+ balance was negative during most of the 23 hr period, the Na^+:K^+ ratio in the 24 hr salivary collections was about 7 and the excretion of Na in the urine was negligible.

When, in section *c* of Fig. 4, the concentration of $NaHCO_3$ solution offered for 1 hr each day was changed to 952 m-equiv/l., the voluntary intake increased and the external balance became positive for a significant period of each day. On the fourth day the animal drank a large amount and the Na^+:K^+ ratio of the next 24 hr specimen of saliva was normal and the kidneys excreted 181 m-equiv of Na. On the fifth day the sheep drank very little Na solution, and the record of sampling episodes reflected this lack of interest in the presence of a positive Na^+ balance. The average voluntary intake for the period was 624 m-equiv Na/day.

In period *d* of Fig. 4 the concentration of $NaHCO_3$ solution was reduced to 119 m-equiv/l. On the first day the number of sampling episodes increased but the intake was small. On the second day the number of sampling episodes was the largest for the experiment and the amount drunk was almost adequate to return the Na^+ balance to equilibrium. This

involved drinking over 3 l. of the Na solution within the hour. During the remainder of the period the voluntary intake approximated the amount of Na^+ deficiency and thus was just adequate to return the external balance to equilibrium at the beginning of each day. The over-all state of Na^+ deficiency prevailing was reflected in the low salivary Na^+:K^+ ratio (approx. 3·0) and the complete renal conservation of Na. The fact that the animal was maintaining a Na^+ equilibrium involving a considerable residual deficiency is reflected also in the decreased Na^+ loss during the 23 hr. The reduced salivary Na^+:K^+ ratio caused the Na^+ loss to be 200–300 m-equiv/day less than in section *c* when the animal was drinking the 952 m-equiv $NaHCO_3$/l. solution. The average voluntary intake for the period was 313 m-equiv Na/day. The increased number of sampling episodes during this period of 119 m-equiv $NaHCO_3$/l. was associated with some clear-cut changes in the animal's behaviour, which will be described in detail in a subsequent paper dealing with conditioned salivary reflexes associated with Na appetite.

In period *e* of Fig. 4, on the first day the animal had access to the $NaHCO_3$ solution at 476 m-equiv/l. concentration, it drank immediately 3·28 l. = 1561 m-equiv Na. It sampled the solutions much less on the following day but in an initial episode it drank 1·26 l. = 600 m-equiv of Na. The very large Na^+ intake, greatly in excess of deficit, resulted in a large positive Na^+ balance. The salivary Na^+:K^+ ratio returned to normal and there was a large urinary Na excretion which persisted throughout the period. During the period the balance was persistently positive, though it approached Na^+ equilibrium after 23 hr of salivary loss. No measurements were made which might indicate whether the excess Na^+ in the body was as an increased amount of intrarumenal Na, an increased extracellular Na or as storage in a depot such as bone. The average number (10) of sampling episodes for the period was considerably less than the preceding period. The average voluntary intake for the period was 784 m-equiv Na/day.

Finally, in period *f*, the animal was permitted access to the 476 m-equiv $NaHCO_3$/l. during the whole day, and the intake was recorded also during the usual 1 hr period. A small amount was drunk during the hour, the over-all intake for the period (848 m-equiv Na/day) approximating that during the preliminary control period (*a*), when the solution was 238 m-equiv $NaHCO_3$/l. The animal remained in positive Na^+ balance, the salivary Na^+:K^+ ratio was normal, and there was a large urinary Na excretion.

It is convenient here to record one further aspect of the drinking behaviour in the experiment of Fig. 4. Figure 5 shows the percentage of the intake drunk during each 5 min period of the hour during which each

concentration was offered. The principal fact emerging is that with the 952 m-equiv/l. concentration the animal took 90 % of the total within 5 min, and virtually all within 15 min. From 15 min onwards it drank considerable amounts of water. The same pattern held with 476 m-equiv $NaHCO_3$/l. except that no water was taken during the hour. By contrast,

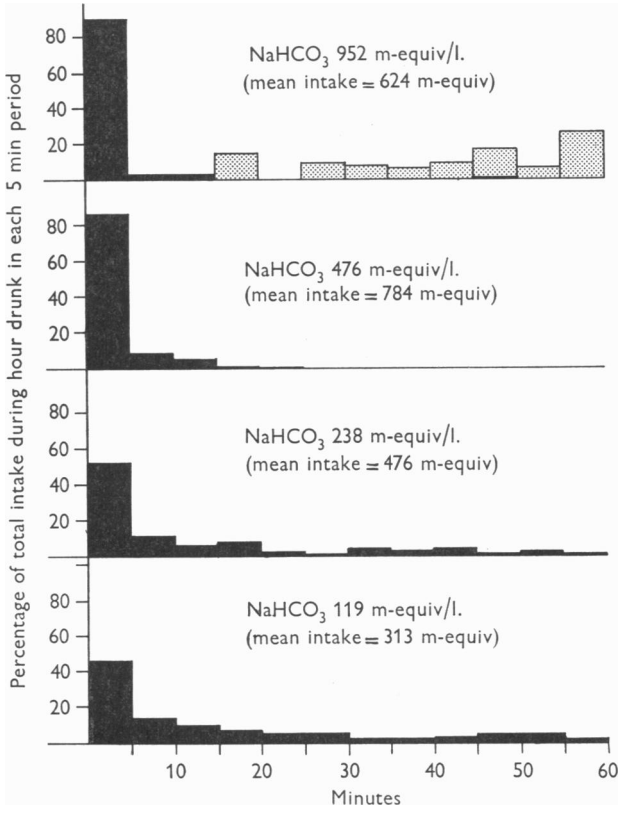

Fig. 5. P.F. 33, Merino cross-bred ewe. The percentage of the voluntary intake drunk in each 5 min period of the hour of access at the various concentrations at which the $NaHCO_3$ solution as offered (▤ H_2O, ■ $NaHCO_3$). The figure shows also that only with the 952 m-equiv/l. solution was there any voluntary intake of water during the hour. The average of the 5 days in each of periods *b*, *c*, *d*, *e* of Fig. 4 is shown.

with the 119 m-equiv/l. concentration only 44 % of the intake was taken in the first 5 min, and drinking continued over the whole hour. This was seen also with the 238 m-equiv $NaHCO_3$/l. and in neither case was any water drunk during the hour.

A study on Ned similar to Fig. 4 extended over 67 days divided into periods of approximately 7 days. With 238 m-equiv $NaHCO_3$/l. available all day, the voluntary intake was 239 m-equiv/day. When the same

solution was available for 1 hr/day only, the average intake was 243 m-equiv. With 952 m-equiv/l. the average intake in the hour was 362 m-equiv, and with 119 m-equiv/l. the average intake was 100 m-equiv. After a 7-day period with the 119 m-equiv $NaHCO_3$/l. available all day (intake equalling 279 m-equiv/day) average intake by the animal when the solution was available again for 1 hr only was 223 m-equiv. With 476 m-equiv/l. the average intake was 329 m-equiv, and when this solution was available all day the average intake was 351 m-equiv; and with 952 m-equiv/l. available all day, the average intake was 426 m-equiv/day. The pattern of voluntary intake over the hour of access resembled that recorded for P.F. 33 (Fig. 5). A detailed study was conducted on T.P. 12 during which the three usual electrolyte solutions were available for 15 min only. During a control period, when the solutions were presented every third day, the observations confirmed those reported above for 420 m-equiv Na/l. solutions. Following this the concentration of the Na solutions was varied from 220 to 880 m-equiv/l. Though this was the sheep's first experience of an experiment involving variation of concentration, the total intake remained approximately constant, and the preference for $NaHCO_3$ was retained. For example, one day it drank 2·61 l. of the 220 m-equiv Na/l. solutions in the 15 min, whereas on the next day, when the concentrations were increased fourfold, the volume drunk was 0·56 l. In both instances about 75% of the intake was as $NaHCO_3$ and the sheep rejected KCl throughout the 32 days of the experiment.

Summarizing, the results showed that when Na solutions were presented for a very short period only the animals usually drank enough to repair most of the Na^+ deficit caused by fistula loss. Under these conditions they showed a considerable capacity to vary the volume drunk when the concentration of the Na solutions was changed, so that intake remained approximately constant. The sheep became very excited when presentation of the Na solutions was imminent, and the bulk of the voluntary intake occurred during the first 5–10 min of access, following which there was a decline of interest in the solutions.

DISCUSSION

In the experiments where normal sheep were offered the salt solutions to drink some animals had a large voluntary intake though there was clearly no deficiency of Na^+. Generally, there was a preference for NaCl over $NaHCO_3$. The concentration of the Na solutions offered was 420 m-equiv/l. and in some instances the amount of water drunk was such that the Na^+ concentration of the daily fluid intake was greater than that of blood. Thus the voluntary Na^+ intake resulted in a very large urinary

excretion of Na at concentrations as high as 380 m-equiv/l. There was considerable variation within the small group of animals studied. The results were consistent with Richter's (1956) findings of liking for salt in the absence of metabolic need. However, unambiguous evidence that this behaviour in sheep was not due to previous experience depends on the outcome of experiments with sheep whose previous history in relation to salt access is known with certainty.

The effect of making a parotid fistula which caused loss of Na^+ was clear-cut. In the sheep which had a small voluntary Na intake beforehand there was a large increase, and usually this involved a preference for $NaHCO_3$ over NaCl. In the instance of the sheep which had a large Na intake before operation there was usually an increase in the proportion of $NaHCO_3$ drunk. There was one exception (P.F. 43) which after 4 days of Na depletion drank 3·46 l. of NaCl, and in the following month always drank NaCl and rarely drank much $NaHCO_3$ though it often tasted it. All sheep with long experience of self-selection, e.g. P.F. 33, P.F. 40, P.F. 48 and T.P. 12, showed a preference for $NaHCO_3$, although they frequently drank a significant amount of NaCl every few days. This preference was less apparent when the animal had been Na-depleted for 2–4 days and showed a sense of urgency and was very excited by the impending presentation of the Na solutions. The selection of a predominance of $NaHCO_3$ over NaCl was a more appropriate choice in the face of the biochemical distortion produced by the predominantly $NaHCO_3$ loss from the parotid fistula. The mechanism of selection has not been investigated. One possibility is that the fall in pH and HCO_3 concentration of the arterial blood perfusing the taste buds is in some way responsible, an hypothesis it should be possible to test by making appropriate infusions into the carotid artery loop supplying a 'vascularly isolated' tongue during the act of self-selection (Denton *et al.* 1959). An alternative possibility is that in the course of random trial and error drinking of the solutions, the animal took enough $NaHCO_3$ to produce subsequently a particular sense of alleviation of need, and that retroactive association of benefit and taste established a preference for that solution.

The quantitative study of voluntary intake in relation to degree of Na^+ deficiency showed that, when the solutions were available all day, many animals maintained themselves in normal balance with a normal salivary Na^+:K^+ ratio. Others maintained an oscillation of balance involving some residual Na^+ deficiency as shown by a salivary Na^+:K^+ ratio of 3–10, and little or no urinary Na excretion. A striking finding was the ability of the sheep to adjust Na intake to need by varying the volume of Na solution drunk per day in the face of change of Na^+ concentration of the solution offered.

This self-regulating ability was clearer from studies in which the solutions were available for 15–60 min only each day or on every second or third day. The external balance studies showed that generally the amount of Na solution drunk in the hour was adequate to replace the Na^+ deficit during the previous 23 hr. The study on P.F. 33 (Fig. 4) indicates that judgement of voluntary intake was usually accurate. This returned the Na^+ balance to equilibrium but, as Na^+ loss continued during the next 23 hr, the balance was negative during most of the day, a finding reflected by low salivary Na^+:K^+ ratio and urinary Na^+ conservation. If, because of an episode with very large Na^+ intake, there was a large positive balance, the intake was usually reduced next day. On the other hand, once the positive balance was established, sometimes the animal maintained a status involving fluctuation of balance on the positive side of Na^+ equilibrium, an observation consistent with the primary finding that Na^+-replete non-fistulated sheep will drink the solutions. The findings in this respect were somewhat suggestive that rate of change of Na^+ balance in the preceding period was influential in determining drinking behaviour as well as the apparent primary determinant of the actual state of Na^+ balance. Consideration of the balances and the increased intake when there were 2–3 days of depletion highlights one other fact shown graphically in Fig. 5. As much as 80–90 % of the intake occurred during the first 5 min of access to the solution. The findings are suggestive that the action specific energy (Lorenz, 1950; Tinbergen, 1951; Haldane, 1956) for Na appetite is accurately determined by metabolic balance. The specificity of the appetite for Na solutions was confirmed frequently by the rejection of food, H_2O or KCl by the restless and excited animal which continued to bleat and jump up and down until Na was offered. The specificity of the appetite was also clear from multiple analyses of the rejection and acceptance pattern by the animal when the four fluids were presented. The fact that this action-specific energy was discharged to a large extent in most instances by a single drinking act within the first 5 min of access to fluid raises the fascinating question as to how the relevant information on satiation is computed by a postulated neural centre determining appetite. For example, with T.P. 12 the animal had had no previous experience of variation of Na^+ concentration of the offered fluid until this occurred under the régime of access for 15 min only. Yet on the different days when it met solutions of NaCl and $NaHCO_3$ of 220 and 880 m-equiv/l. for the first time it immediately adjusted the volume drunk so that the total intake was approximately equal at the two differing concentrations. The question whether drinking (presumably a satiating consummatory act (Tinbergen, 1951)) is eventually inhibited as a result of an adequate number of taste bud impulses coupling centrally with an adequate number of oesophageal

'metered' swallowing acts turns on appropriate future experiments on animals with oesophageal fistulae. There seems no possibility that absorption into the blood would be rapid enough to raise the question of this inhibiting the act of drinking (Le Magnen, 1953).

Considering the cause of the Na appetite of deficient sheep, two of the possibilities are that either the increase of aldosterone concentration in blood or the changed composition of saliva in the mouth caused by aldosterone secretion (Denton *et al.* 1959, 1960) stimulate this behaviour. However, normal self-regulatory capacities have been observed in a large number of bilaterally adrenalectomized sheep with parotid fistulae which have been maintained in our laboratory by the self-selection régime. Voluntary ingestion of $NaHCO_3$ adequate to correct deficiency has been seen at times when there was no residual action from electrolyte-active steroid such as DOCA as shown by the secretion of parotid saliva of normal composition despite Na^+ depletion. These observations are evidence against either of the above possibilities as a stimulus of Na appetite. In relation to the spontaneous ingestion of salt solution by sheep which are demonstrably in normal Na^+ balance, a finding which seems to have been confirmed by many field observations, it seems probable that the innate releasing mechanism is the taste of salt encountered during exploratory tasting. The basis of the fairly widespread propensity to ingest salt upon encountering it lies presumably in the selection of an inherited pattern conferring survival advantage on the species. Na^+ deficiency could occur under a variety of natural conditions particularly in ruminant animals (Denton, Wynn, McDonald & Simon, 1951; Denton, Goding, Sabine & Wright, 1958). Notwithstanding the variation of voluntary intake seen in the Na^+-replete animal, Na^+-deficient animals, with very few exceptions, show an active appetite for Na.

If one considers the thirst mechanism, there appears to be evidence that the action-specific energy for drinking determined by osmotic pressure changes (Wolf, 1958) depends upon a fairly precisely defined region of the hypothalamus (Andersson, Jewell & Larsson, 1958) and is reinforced by proprioceptor impulses from the dry mouth and pharynx. There is much less evidence on a physicochemical basis of action-specific energy for Na appetite than there is in the case of thirst. With thirst, Wolf (1958) has shown that raising the osmotic pressure of the blood by 1–2%, a degree of change shown by Verney (1947) to release antidiuretic hormone, causes ingestion of water. We have produced the same effect on sheep by slow intracarotid infusion of 4 M-NaCl or 2·7 M glucose, and Andersson *et al.* (1958) have shown that stimulation of a specific region of the mid hypothalamus by injection of 0·1 ml. of 1·5–2% NaCl causes compulsive drinking. With Na appetite, during Na^+ deficiency it is possible that the

accumulation of action-specific energy is determined directly by Na^+ depletion of the specific cells in the central nervous system controlling this appetite. Alternatively, as with the intimately linked problem of control of aldosterone secretion (Farrell, 1958; Denton *et al.* 1959, 1960; Bartter, Mills, Biglieri & Delea, 1959; Coghlan, Denton, Goding & Wright, 1960), a number of peripheral proprioceptor mechanisms (e.g. vascular stretch receptors) might be postulated as the main receptor mechanisms controlling appetite, or secondarily as despatching impulses modifying the activity of the centre postulated above. In the sheep experienced in this regard it has been demonstrated that voluntary Na^+ intake may closely approximate the degree of deficiency. We have referred already to the proposition that the consummatory act of drinking is inhibited at the appropriate point by proprioceptor impulses discharging the action-specific energy. Future careful studies of fistulated animals on the first occasion that they encounter the Na solutions may reveal whether intake is accurately related to degree of deficiency initially, or whether it is retro-active effect from satiation experience which leads ultimately to precise quantitative adjustment of appetite.

If the receptor system determining Na appetite is within the brain or the circulatory system an outstanding problem remaining is how the consummatory act of Na drinking during 3–4 min discharges the action-specific energy and causes a precipitate decline in motivation long before the ingested material could repair the cellular, circulatory, or blood composition abnormalities which may determine appetite. A similar problem exists, of course, in the capacity of the water-depleted camel to correct a deficiency of 50–100 kg in a single drinking act (Schmidt-Nielsen, 1959), but the phenomenon is more complicated in the Na^+-deficient sheep in view of the ability to adjust volume drunk to the concentration of the $NaHCO_3$ solution presented.

SUMMARY

1. Some sheep when offered solutions of Na salts to drink voluntarily ingested considerable amounts though they were in normal Na^+ balance.

2. The establishment of a permanent unilateral parotid fistula caused loss of 1–4 l. of alkaline saliva/day containing 180–700 m-equiv of Na, mainly as $NaHCO_3$. There was a large increase of appetite for Na. The voluntary intake of many animals was adequate to maintain a normal Na^+ balance whilst others oscillated around a balance involving some degree of residual Na^+ deficiency. Offered a choice between NaCl and $NaHCO_3$ these animals showed a clear preference for $NaHCO_3$.

3. If the fistulated sheep were offered continuous access to $NaHCO_3$ solution and water, and the concentration of the $NaHCO_3$ solution was

varied from 119 to 940 m-equiv/l., the animal varied the volume drunk so that Na intake remained relatively constant and approximated the loss from the fistula.

4. If the Na solutions were available to the fistulated sheep for only 15–60 min each day, the animal usually ingested enough to correct its deficit. 80–90% of intake occurred during the first 5 min. When the concentration of Na solution was increased, the volume drunk was decreased proportionately. If access were permitted only on every 2nd or 3rd day, the amount of Na^+ ingested was increased commensurately.

6. In experiments where solutions were offered for a short period only there was a large increase in frequency with which solutions were sampled during Na^+ deficiency. The sheep rarely ingested any KCl solution when it was included amongst the solutions offered. The preference for $NaHCO_3$ over NaCl was maintained during this régime of short-term access.

7. The experimental results have been discussed in terms of concepts of behaviour advanced by ethologists. The action-specific energy for drinking solutions of Na salts appears in the Na^+-deficient animal to be related to the degree of Na deficit, and the problem arises, as in more simplified form with thirst, how the consummatory act of drinking is inhibited. The hypothesis is advanced that there is a central integration of taste impulses from the tongue signalling concentration with pharyngo-oesophageal proprioceptor impulses signalling volume swallowed. The problem remains as to how the consummatory act discharges the action-specific energy long before the ingested material could repair any physiological consequence of Na^+ deficit either within the *milieu intérieur*, or the intracellular fluid of a neural centre controlling Na intake.

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