A STUDY OF THE INFLUENCE OF NITRITE OF SODIUM ON ANIMAL METABOLISM¹

By N. F. SURVEYOR, M.A., B.Sc., M.D. (*Bombay*), M.R.C.P. (*London*),
D.P.H. (*Cambridge*), *Honorary Physician, J. J. Hospital, Bombay.*
From the F. D. Petit Laboratory, Grant Medical College, Bombay.

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The nitrites, both organic and inorganic, have received considerable attention since the time that Sir Lauder Brunton recommended the use of amyl nitrite for cutting short an attack of angina pectoris on purely physiological grounds. Sodium nitrite has been used for the same purpose, or better still, for preventing the onset of an attack of this disease, and also in asthma, on the supposition that the latter disease is produced by the spasmodic contraction of the smaller bronchioles. Again, recently nitrites have been recommended in hæmoptysis, the idea being to produce dilatation of the peripheral blood vessels and thus lessen the amount of blood going to the lungs. However, the present investigation has been undertaken not for the purpose of studying this action of sodium nitrite, but to see whether any change is produced in animal metabolism by subcutaneous use of the drug.

Petrone and Darschkewitsch claim that in syphilis and tabes subcutaneous injection of sodium nitrite in gradually increasing doses produces considerable amelioration of the condition of the patient, the dose varying between 0·01 to 0·06 gram per diem. The favourable result is attributed to the antisyphilitic and antiseptic properties of the drug. It seems too much to expect much antiseptic effect from such small quantities of a comparatively mild antiseptic like sodium nitrite. As for the specific antisyphilitic property, the explanation is stating the result in other words, and does not bring us nearer the mode of action of the drug. It appeared that perhaps a study of

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the metabolic changes occurring in the dog under the influence of the drug might show some results which may bring us nearer the truth.

Four such observations have been made, and the last series of observations were continued for a sufficiently long period to show any change that might be produced. The following is the general plan that was adopted in these observations :—

The animal was accustomed to live in a cage specially designed for these experiments, and its weight was observed for a few days before the experiment was started. As soon as the weight remained constant it was kept on a particular diet ; this consisted in all cases of 100 grams of Spratt's dog biscuits powdered and mixed with 100 grams of sterilised minced meat, with a known quantity of distilled water, viz., 200 c.c. The amount of water in the meat was estimated.

The fæces and urine were analysed for water, fat, and nitrogen.

The fat was estimated by Soxhlet's method, while Kjeldahl's method was employed for nitrogen estimation. Urea in urine was estimated with hypobromite of soda.

The weight was observed daily to see whether the animal was in metabolic equilibrium or not. As the animals did not have a motion every day, it is necessary to take the average result of the different periods for the purpose of comparison. The drug used was Merck's purified sodium nitrite sticks suitable for injections. It was dissolved in sterilised distilled water. The dose given was 1 c.c. of 1 per cent. solution for Experiment I, while in the other three experiments it was 1 c.c. of a 3 per cent. solution, and in the second series of injections in the last experiment (IV) it was 2 c.c. of 3 per cent. solution. This last amount is equivalent weight for weight to 9 grains of sodium nitrite given hypodermically to a full grown man. This last series was continued for four days, however no evil effect was noticed in the dog even temporarily.

The principal facts noticed during these experiments were that all the animals slowly increased in weight during the period of

observation, and that none of them went off its feed or became restless.

For the purpose of comparison the daily tables are not satisfactory as, although the animals were thoroughly accustomed to live in the experimental cage, occasionally, for some unknown reasons, a marked daily variation has been produced in the urinary secretion ; however, on taking an average of each period, these variations have become very slight.

Thus the averages of the amount of urine passed in twenty-four hours show a distinct increase in all except in Experiment IV, first series, which shows a diminution. This is probably due to some experimental error, in as far as on the first day of the normal period the animal voided a large amount of urine—perhaps through some oversight the dog had an extra amount of water on the preceding day. On the second and third days the amount fell steadily, and if we take the averages of these two days in conjunction with the quantity of water in the fæces, the result is the same as in the other three experiments, *i.e.*, the total of the urinary secretion and the water in the fæces is much larger during the period of drug administration. Again, if we take the averages of all normal periods as far as the amount of urinary secretion is concerned, and compare them with those of the nitrite period in Experiment IV, we find that the amount of water is increased during the latter. The increase is about 5 per cent., while if the first normal day is excluded the increase is about 8 per cent. during the nitrite period.

Most of the authorities I have been able to refer to are of opinion that nitrites have no marked effect on urinary secretion. The slight increase in the amount of fluid noted by them is said to be due to the dilatation of the blood vessels of the kidneys. None believes that there is any increase of urea or total nitrogen. Cushny and Sollman, in their works state that the urine may be increased or diminished according as the dilatation of the blood vessels of the kidneys preponderates over that of the general peripheral vessels or *vice versa*.

In my experiments the increase of water noticed in almost all the cases is not less than 10 per cent and not higher than 32 per cent. ; in

Experiment IV the first series forms an exception, but, as stated above when the first day of the first normal is not counted, the increase is about 8 per cent on the whole experiment.

Excretion of urea and nitrogen is also fairly increased in all the cases without exception ; in Exp. I the animal had a very small dose and the increase is also slight. This at least indicates something more than simple dilatation of the vessels, again these effects are partly kept up during the succeeding normal periods which also indicates something more than mere vaso-dilatation. The increase of urea is neither less than 8 per cent. nor greater than 26 per cent., while the increase of nitrogen varies between 7 and 24 per cent.

As regards the absorption of water from the alimentary canal, Exp. I, II, and III show an increase to the extent of 15 to 20 per cent., on the other hand, the percentage of solids as compared with that of water was also increased in these three experiments. In Exp. IV the last series of observations as compared with the last normal shows the same result. This dog was probably less susceptible to the action of the drug as a larger dose produced results similar to those of the other experiments, besides it is probable that the animal was not in metabolic equilibrium in the commencement of the experiment, thus the amount of urine passed on the first day was considerable as has been stated above.

As regards the question of absorption of nitrogen and fat, Exp. II, III, and IV (with the larger dose) show a distinct increase, while Exp. I and IV (with the smaller dose) show a diminution, this may be due to the extremely small dose in Exp. I, and to the insusceptibility of the last animal. The increase of nitrogen absorption varied between 7 to 14 per cent., while that of fat varied between 9 to 21 per cent.

As regards the explanation of the various effects observed on the urinary secretion and absorption of food stuffs from the alimentary canal we may suppose that the dilated state of the renal vessels might be strong enough to produce the increased flow of water, but it is not quite enough to account for the increase of total nitrogen and urea. This certainly seems to show something more than mere dilatation of the blood vessels. This may be due to the greater excretory action of

the renal epithelium only or to increased functional activity of the liver. To support the latter view we may note that the nitrites produce temporary glycosuria (Cushny and Sollman).

During the period of drug administration we have seen that the amount of fæces was diminished—this may be due to interference with the peristaltic action of the bowels. Vapour of amyl nitrite is said to lessen considerably the activity of unstriated muscles, as in the case of blood vessels, intestines, and ureters (Cushny).

Thus on account of this effect on the muscles of the intestines it is likely that the food remained in the canal for a longer while and thus greater absorption of nitrogen, fat, and water took place as we have found in the experiments.

The dilating effect of this drug on the blood vessels is only temporary even when the drug is given by mouth—and the dose is about 2 grains. Larger doses produce symptoms of collapse and gastric irritation, the latter being due probably to the setting free of nitrous oxide by the action of the HCl of the gastric juice.

In case of hypodermic use, the dilating effect is also temporary and not much perceived even after a dose of 3 grains, while on the other hand the reflex irritability of muscles is distinctly lowered and the effect is fairly permanent, thus exaggerated knee-jerk and ankle-clonus become much less marked.

Again, the hypodermic method is perfectly harmless, no local pain or swelling is noticed, and the organism can stand much larger doses. The average dose of 0.05 to 0.5 gram divided into two portions per day has been found quite safe (*Medical Annual*, 1897, p. 554).

These facts coupled with the results of the experiments mentioned above show that this drug has other properties besides that of producing dilatation of blood vessels. That tissue change and absorption of food stuffs are affected by nitrites is almost certain, as the hypodermic use of nitrites, particularly amyl nitrite, has been found to be followed by glycosuria in some observations (Cushny and Sollmann).

All these facts seem to show that the beneficial influence, if any exists, of the drug in syphilis, and especially nervous affections following syphilis, is probably due to the improved nutrition of the organism.

It is a curious fact observed clinically in my cases that the drug is comparatively less efficient in dilating the blood vessels when injected subcutaneously as compared with its action when taken *per Os*. Thus an injection of 3 grains produces very slight fulness of the blood vessels, while the same dose by mouth produces symptoms of collapse in some cases. Is it not probable that the rapid dilatation produced by this drug when given by mouth may be due to the liberation of nitrous oxide by the gastric juice and the subsequent absorption of the gas? On the other hand, this decomposition cannot take place in the alkaline tissues of the animal organism, and the milder effect is due to the undecomposed drug. It has been stated by Browning (*Therapeutic Gazette*, 1901) that the effect of the drug is temporary when injected subcutaneously as it is rapidly eliminated from the system; but this is not the fact, as its presence can be detected in the urine even after 24 hours in a person who had an injection of 3 grains on the previous day.

My best thanks are due to the Government of Bombay for having given me a grant for this research.

EXPERIMENT I. SHOWS THE AVERAGES OF EXCRETION OF DOG I

DAYS	URINE			FÆCES			
	Quantity c.c.	Total urea grms.	Total nitrogen grms.	Total quantity grms.	Total solids grms.	Total water c.c.	Solids % Water %
Averages of four normal days ...	124	4.94	2.61	48	12.99	35.01	27.06 72.94
" " four sodium nitrite days ... (1 c.c. of 1 % solution)	136	5.31	2.81	45.97	15.12	30.85	67.10 32.90
" " four days following treatment	139	5.97	3.00	49.64	14.93	34.70	69.92 30.08
							0.84 0.84
							1.07 1.07

EXPERIMENT II. SHOWS THE AVERAGES OF EXCRETION OF DOG II

Averages of three normal days ...	100	8.38	3.88	53.35	17.10	36.45	31.92 68.08
" " four sodium nitrite days ... (1 c.c. of 3 %)	132.5	10.17	4.68	44.35	14.22	30.14	32.06 67.94
" " two days following treatment	135	9.60	4.43	33.50	10.45	22.55	67.31 32.69
							0.62 0.62
							0.74 0.74

EXPERIMENT III. SHOWS THE AVERAGES OF EXCRETION OF DOG III

Averages of three normal days ...	122	7.95	3.56	39.63	14.33	25.30	36.15 63.85
" " three sodium nitrite days ... (1 c.c. of 3 % solution)	152	8.62	4.02	33.06	12.75	20.31	38.58 61.42
" " four days following treatment	170	10.47	4.34	38.47	12.24	26.23	66.52 33.48
							0.78 0.78
							0.84 0.84
							1.06 1.06

EXPERIMENT IV. SHOWS THE AVERAGES OF EXCRETION OF DOG IV

DAYS	URINE			FAECES						
	Quantity c.c.	Total urea grms.	Total nitrogen grms.	Total quantity grms.	Total solids grms.	Total water c.c.	Solids %	Water %	Total nitrogen grms.	Total fat grms.
Averages of three normal days	...									
"	163	7.70	3.78	28.55	9.95	18.60	34.87	65.13	0.46	0.75
" four sodium nitrite days (1 c.c. of 3 % solution)	147	8.38	4.26	41.69	13.12	28.57	31.49	68.51	0.67	0.89
"	150	9.90	4.84	32.28	11.44	20.84	35.43	64.57	0.62	0.80
"	175	10.36	4.94	32.52	11.31	21.21	34.79	65.21	0.58	0.71
"	140	8.72	4.77	40.01	12.55	27.46	31.36	68.64	0.73	0.71

EXPERIMENT III. SHOWS THE DAILY EXCRETION OF DOG III

Normal day	...	...	...	1	110	7.35	3.36	56.92	19.58	37.34	34.40	65.60	1.17	1.17
" "	...	...	...	2	80	5.77	2.73	...	...	...	...	...	...	...
" "	...	...	...	3	175	10.73	4.58	61.97	23.40	38.57	37.74	62.26	1.60	2.00
Sodium nitrite day (1 c.c. 3 % sol.)	4	110	7.65	3.53	...	...	...	...	...	...	...	...	...	...
" "	5	180	9.10	4.61	46.74	20.14	26.60	43.10	56.90	1.35	1.44	1.44	1.44	1.44
" "	6	165	9.10	3.93	52.44	18.16	34.28	34.60	65.40	1.16	1.05	1.05	1.05	1.05
" "	7	190	10.06	4.11	...	...	...	...	...	...	...	...	...	...
Normal day following treatment	8	180	10.46	4.38	76.60	26.16	50.44	34.15	65.85	1.74	1.73	1.73	1.73	1.73
" "	9	150	10.20	4.33	...	...	...	...	...	...	...	...	...	...
" "	10	160	11.14	4.56	69.30	22.80	46.50	32.90	67.10	1.38	1.38	1.38	1.38	1.38